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Functional Organization Of Sympathetic Cardioregulatory Pathways In The Medulla And Spinal Cord Of The Cat

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FUNCTIONAL ORGANIZATION OF SYMPATHETIC CARDIOREGULATORY
PATHWAYS IN THE MEDULLA AND SPINAL CORD OF THE CAT

by

James Lorne Henry
Department of Physiology

Submitted in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Ontario

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ABSTRACT

This study was done to localize the sites of origin in the medulla of fibres projecting monosynaptically to sympathetic cardio-acceleratory neurons in the spinal cord by recording evoked antidromic responses from the medulla during electrical stimulation of the terminals of the descending fibres. To ensure selective activation of terminals of descending cardioacceleratory fibres the distribution of sympathetic neurons in the thoraco-lumbar intermediolateral nucleus (ILN) and the precise localization of cardioacceleratory neurons within the ILN were determined.

Serial transverse sections of the spinal cord of eight adult cats were stained with thionin and the topography and numerical distribution of ILN neurons were obtained. The ILN extends from C₈ - T₁ to L₄ and is located in the lateral horn of the grey matter except in segments C₈ - T₁ and L₁ - L₂ where the neurons are scattered throughout finger-like projections of grey matter. Total neuron counts on left and right sides were not significantly different. A statistically significant ($p < 0.02$) difference, however, was demonstrated between the mean counts in four female ($35,543 \pm 1,411$) and four male ($45,765 \pm 2,556$) cats. The number of ILN neurons varies from segment to segment, with the highest counts in segments T₁ - T₂ and L₃ - L₄.

The modal value of the estimated cross-sectional area of neurons in one male cat was $290 \mu\text{m}^2$.

The distribution of cardioacceleratory neurons in the ILN was determined by observing the effects on heart rate of electrical stimulation of histologically verified sites within this nucleus. In 20 adrenalectomized cats with bilateral vagotomy and spinal transection (C_7) stimulation of the ILN on the right side of segments T_1 to mid- T_8 elicited cardioacceleration and arterial hypertension at 84 sites. These responses were not affected by administration of gallamine triethiodide (5 mg/kg). Administration of propranolol (2 mg/kg) abolished the cardioacceleratory but not the pressor response. Cardioacceleratory responses on the left side were significantly ($p < 0.05$) smaller than those on the right. Maximum cardioacceleration was obtained at a stimulus frequency of 25 Hz, maximum arterial hypertension at 20 Hz. The greatest change in heart rate for a given change in frequency occurred in the range 0-5 Hz; the greatest change in arterial pressure occurred between 0 and 20 Hz.

In 22 cats evoked responses were recorded from 266 medullary sites in 216 penetrations during electrical stimulation of histologically verified cardioacceleratory sites in the ILN in T_2 . Responses were likely non-synaptic because they followed frequencies above 200 Hz and were unaffected by hypoxia and barbiturate overdose. Responses were presumed to be from cell groupings because they showed the characteristic shift of peak latency as responsive regions were approached and sites

of recording corresponded to specific medullary nuclei. The stimulus likely selectively activated descending autonomic fibres because stimulus sites were verified histologically within the ILN, displacement of the electrode 0.5 mm eliminated the medullary response and fibres adjacent to the ILN are propriospinal. It is concluded that the evoked responses represented antidromic invasion of neurons whose terminals were activated in the ILN. Therefore, medullary structures from which responses were recorded project monosynaptically to the ILN. Four sites in 27 penetrations in the contralateral medulla demonstrate that crossing-over occurs but that descending fibres are predominantly ipsilateral.

Electrical stimulation of 81 responsive sites elicited cardioacceleration from 17 sites in the N. lateralis reticularis and N. parvocellularis and cardioinhibition from 29 sites in the raphé NN., the N. paramedium reticularis and the N. medullae oblongatae centralis. It was concluded that the N. lateralis reticularis and the N. parvocellularis give rise to descending fibres which activate cardioacceleratory ILN neurons and the raphé NN., the N. paramedium reticularis and the N. medullae oblongatae centralis give rise to fibres which inhibit these neurons. Hypotension was elicited from the same structures as cardiac slowing, suggesting that similar mechanisms mediate these responses. Stimulation of cardioacceleratory sites produced inconsistent changes in pressure, suggesting that different mechanisms mediate these responses. The results also demonstrate that cardiovascular reflex

arcs do not converge to a final common pathway from the medulla and that some integration of cardiovascular reflexes occurs at the spinal level.

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"It is not within the power of the properly
constructed human mind to be satisfied.

Progress would cease if this were the case.

The greatest joy in life is to accomplish.

It is the getting, not the having. It is the
giving, not the keeping".

- Frederick G. Banting, 1929.

INTRODUCTION

It is generally accepted that medullary integration of cardiovascular reflexes occurs in a diffuse network of neurons in the medullary reticular formation (Alexander, 1946; Peiss, 1965). However, the analysis of the central integration of these reflexes requires precise information regarding the specific brain stem structures involved and the way in which they operate. In this respect, most of the experimental evidence relates to the central sites of termination of primary afferent fibres involved in control of the cardiovascular system (Cottle, 1964; Humphrey, 1967; Miura & Reis, 1969; Biscoe & Sampson, 1970 a, b), but little information is available regarding the structures giving rise to efferent cardiovascular fibres from the medulla.

In earlier experiments it had been demonstrated that electrical stimulation of a discrete region in the medulla of the cat elicited cardioacceleration by activation of sympathetic and inhibition of parasympathetic fibres to the heart (Calaresu & Henry, 1970). As stimulation of this area elicited evoked activity from sympathetic pre- and postganglionic fibres with a short latency it seems reasonable to suggest that neurons in this area are connected directly to sympathetic cardioacceleratory

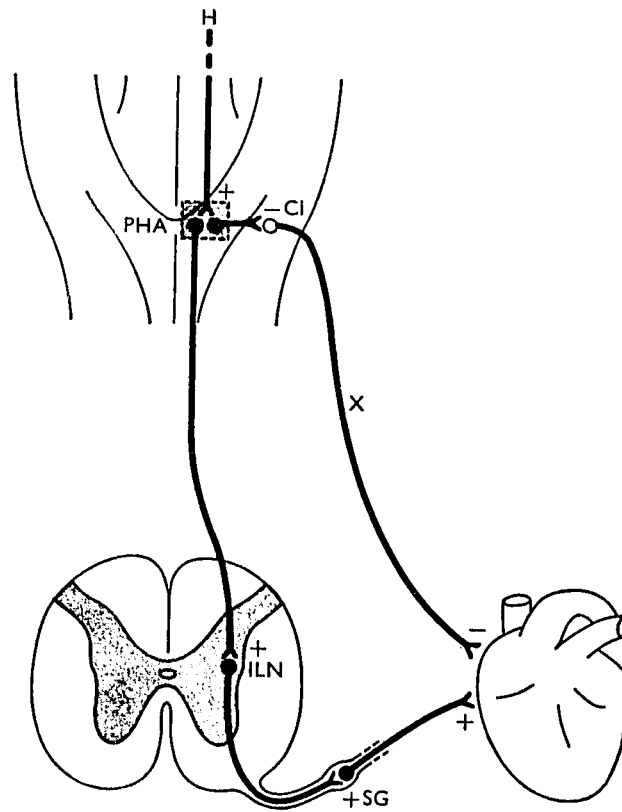
neurons in the spinal cord as shown in Figure 1. A survey of the literature provided no information on the medullary structures projecting to sympathetic preganglionic neurons in the spinal cord. A search was therefore made, using electrophysiological techniques, for medullary structures involved in descending cardioacceleratory pathways to spinal sympathetic cardioacceleratory neurons by activating the terminals of the descending fibres as they enter the thoraco-lumbar intermediolateral nucleus (ILN) and exploring the medulla systematically for sites from which antidromically evoked activity could be recorded.

To ensure activation of the terminals of descending cardioacceleratory fibres, it was first necessary to select the most appropriate region of the spinal cord for stimulation. To do this two projects were completed. The first was to determine the region of the spinal cord in which an electrode would encounter the greatest concentration of sympathetic neurons. Although it is generally accepted that the cell bodies of sympathetic preganglionic neurons are located in the ILN nothing is known of the topography and numerical distribution of these neurons. Therefore, a detailed anatomical study of this nucleus was done to obtain a map of sympathetic preganglionic neurons and an estimate of the number of cells in each segment.

In the second project the segmental distribution of cardioacceleratory neurons within the ILN was determined by observing the effects on heart rate of electrical stimulation

FIGURE 1

Schematic diagram of the suggested connection between a cardioacceleratory area in the medulla and sympathetic cardioacceleratory neurons in the spinal cord (Calaresu & Henry, 1970).



Schematic representation of the proposed functional connexions between the parhypoglossal area and the autonomic nerves to the heart. +, excitatory; -, inhibitory; CI, cardio-inhibitory neurones; H, descending hypothalamic pathway; ILN, intermediolateral nucleus of the thoracic spinal cord, PHA, parhypoglossal area; SG, sympathetic ganglion; X, vagus nerve.

of histologically verified sites within this nucleus.

On the basis of the results of the first two projects it was possible to select the region of the spinal cord for stimulation to activate the greatest number of descending cardio regulatory fibres from the medulla.

HISTORICAL REVIEW

Application of the evoked antidromic technique to the identification of medullary cardioacceleratory structures projecting to sympathetic preganglionic neurons depends on knowing the precise localization of spinal cardioacceleratory neurons. Therefore the first two sections of this review will survey the literature concerning the distribution of these neurons in the spinal cord: the first will describe the anatomical features of the nucleus in which sympathetic preganglionic neurons are found; the second will examine the representation of cardioacceleratory neurons within this nucleus. As the objective of the investigation is to identify the medullary structures projecting to cardioacceleratory neurons the third section will identify the nuclei in the medulla which have been previously implicated in central control of the cardiovascular system.

A. Anatomy of the Intermediolateral Nucleus

The neurons in the thoraco-lumbar intermediolateral nucleus (ILN) of the spinal cord are considered to give rise to the fibres that constitute the sympathetic white rami (Mitchell, 1956). Clarke (1851) first identified the ILN in several species as a column of neurons extending from the spinal accessory nucleus to the lumbar spinal cord. Throughout its length the ILN lies at the border of the grey matter directly lateral or dorsolateral to the central canal in the lateral horn (Crosby, Humphrey & Lauer, 1962). The ILN has been observed to extend as far rostrally as the eighth cervical segment in the cat (Takahashi, 1913; Laruelle, 1937) and in man (Jacobsohn, 1908; Massazza, 1923 a, b, 1924; Bok, 1928; Gagel, 1928, 1932; Greving, 1928; Laruelle, 1937; Riley, 1960), and as far rostrally as the first thoracic segment in the cat (Rexed, 1954) and in the rat (Navaratnam & Lewis, 1970). Caudally it has been observed to end at the level of L₁ (Takahashi, 1913), L₂ (Laruelle, 1937) and L₄ (Rexed, 1954) in the cat. In man it has been observed to end at the level of either L₂ (Gagel, 1928, 1932; Laruelle, 1937) or L₃ (Jacobsohn, 1908; Greving, 1928; Riley, 1960) or to merge with the sacral parasympathetic lateral horn (Bok, 1928; Massazza, 1923 a, b, 1924). In the

rat it has been found to end in $L_2 - L_3$ (Navaratnam & Lewis, 1970).

The neurons of the ILN show a typical distribution of alternating regions of high and low density generally referred to as "beading"; this has been observed in anatomical (e.g. Rexed, 1954) as well as in electrophysiological (Fernandez de Molina, Kuno & Perl, 1965; Polosa, 1967) investigations.

The medial border of the ILN is sometimes difficult to define as the cells merge with those of Lamina VII of Rexed (1952). Laterally, the ILN protrudes out into the white matter giving rise to the typical appearance of the lateral portion of the grey matter referred to as the lateral horn (Rexed, 1954).

B. Location of Cardioacceleratory Neurons in the Spinal Cord

A review of the literature provided no information on the specific localization of cardioacceleratory neurons within the intermediolateral nucleus (ILN) although the segmental distribution of these neurons may be inferred from experiments in which the whole spinal cord was stimulated. Gellhorn, Cortell & Murphy (1946) reported that gross electrical stimulation of the dorsal surface of the spinal cord in the cat in segments C₈ to the most caudal point stimulated in segment T₄ elicited increases in blood pressure, but no attempts were made to determine the topographical localization of cardioacceleratory neurons. Gillespie, Maclaren & Pollock (1970) reported that electrical stimulation of the spinal cord in segments C₇ to T₁₃ in pithed cats and rats elicited an increase in heart rate and in arterial pressure. However, these data provide no information on the specific segmental representation of cardioacceleratory neurons because the exposed tip of the pithing probe that was used as the stimulating electrode extended over several segments and therefore the specific segments from which the responses were being elicited could not be determined.

Further information on the distribution of cardioacceleratory neurons in the ILN may be inferred from the distribution of sympathetic cardioacceleratory nerves to the

heart. Cardioacceleration has been elicited in the cat by electrical stimulation of the stellate and inferior cervical ganglia on the right side (Cannon, Lewis & Britton, 1926) and in the dog by electrical stimulation of the following nerves on the right side: the dorsal and ventral ansae subclaviae and the second, third and occasionally the fourth rami communicantes (Mizeres, 1958).

Finally, the distribution of cardioacceleratory neurons in the spinal cord may be inferred from anatomical investigation of the sites of termination of sympathetic nerves. Cardiac fibres have been reported to originate only from the stellate and cervical ganglia in the cat (Boehm & Nussbaum, 1875; Langley, 1892; Dogiel & Archangelsky, 1906) or from the cervical and upper five or six thoracic ganglia (Perman, 1924) and in man from the upper five thoracic ganglia (Kuntz & Morehouse, 1930; White, Garrey & Atkins, 1933; Ellison & Williams, 1969).

In conclusion, there is no direct evidence of the segmental distribution of cardioacceleratory neurons within the ILN. Information on this distribution from evidence obtained during experiments in which the whole spinal cord was stimulated, or from evidence of the segmental distribution of sympathetic nerves to the heart is unsatisfactory because the stimulation to the spinal cord was applied to several spinal segments at a time and because tracing the sympathetic nerves peripherally does not account for either the intraspinal courses of the preganglionic fibres or the convergence or divergence of preganglionic fibres onto postganglionic neurons in the sympathetic ganglia.

FIGURE 2

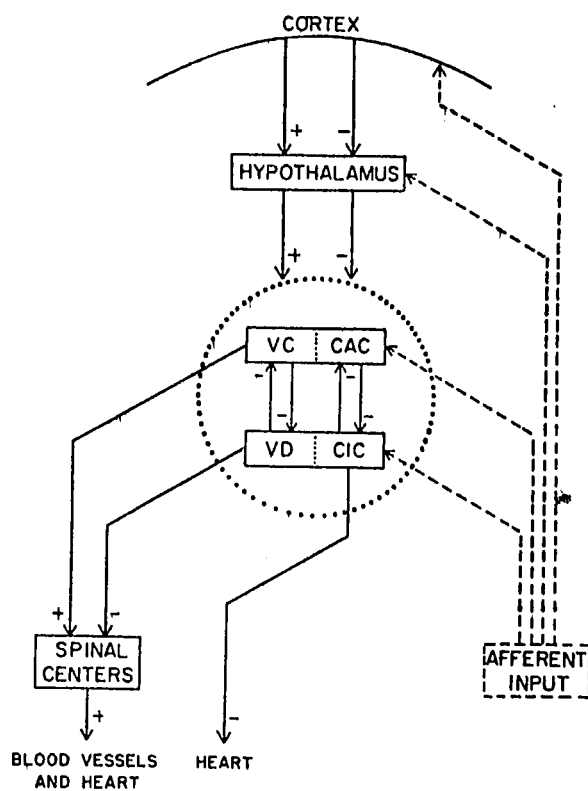
Schematic diagram representing the current concept of central control of the cardiovascular system (Peiss, 1965).

C. Functional Organization of Medullary Structures Involved in Cardiovascular Control

It is generally accepted that medullary integration of cardiovascular reflexes occurs in a diffuse network of neurons in the bulbar reticular formation (Peiss, 1965; see Figure 2). Although experimental evidence for the existence of a vasopressor and cardioacceleratory area in the rostral medulla and of a vasodepressor area in the caudal medulla is available (Wang & Ranson, 1939; Monnier, 1939; Alexander, 1946; Bach, 1952; see Figure 3) the precise location and function of pathways and of cell assemblies involved in cardiovascular control have only recently been investigated.

The objective of this section is to survey the relevant literature on the medullary structures which have previously been implicated in the central control of the cardiovascular system. This information will be later correlated with the structures identified in the present investigation to develop a more complete understanding of the functional organization of the structures involved in medullary cardiovascular reflex arcs.

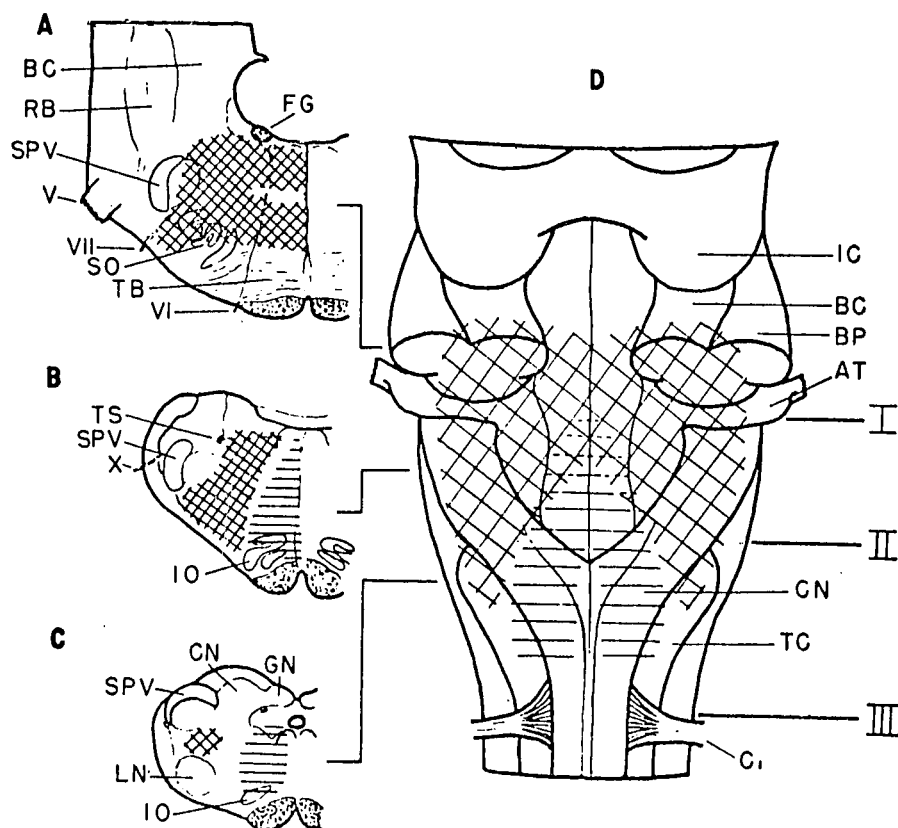
Investigations of the structures involved in medullary control of the cardiovascular system have generally employed one or more of the following techniques: recording from single units whose firing characteristics could be correlated with cardiovascular events; electrical stimulation of specific medullary



Schematic diagram of current concepts of cardiovascular control. *Dotted circle* represents vasomotor area of medulla oblongata. *VC-CAC* indicates vasoconstrictor-cardioaccelerator region; *VD-CIC* indicates vasodepressor-cardioinhibitory region. See text for details.

FIGURE 3

Schematic diagram of the locations
of pressor and depressor centres in
the medulla (Alexander, 1946).



Localization of pressor and depressor centers in the brain stem of the cat. Pressor regions indicated by cross hatching; depressor regions by horizontal ruling. A-C:—cross sections through medulla at levels indicated by guide lines to D; D:—semi-diagrammatic projection of pressor and depressor regions onto the dorsal surface of the brain stem viewed with the cerebellar peduncles cut across and the cerebellum removed. Legend:—AT: auditory tubercle; BC: brachium conjunctiva; BP: brachium ponti; C₁: first cervical nerve; CN: cuneate nucleus; FG: facial genu; GN: gracile nucleus; IC: inferior colliculus; IO: inferior olivary nucleus; LN: lateral reticular nucleus; RB: restiform body; SO: superior olivary nucleus; SPV: spinal trigeminal tract; TB: trapezoid body; TC: tuberculum cinereum; TS: tractus solitarius; V, VI, VII, X: corresponding cranial nerves; I, II, III: levels of transection discussed in text.

structures; recording of evoked potentials; silver impregnation techniques to trace degenerating fibres. Recent developments in the understanding of the central control of the cardiovascular system will be outlined by describing how these four techniques have been applied in determining the central sites of termination of cardiovascular afferent fibres, the intramedullary connections between structures involved in cardiovascular regulation and the sites of origin of efferent cardiovascular fibres from the medulla.

(i) Sites of termination in the medulla of cardiovascular afferent fibres

Silver impregnation techniques have been used extensively in attempts to determine the central sites of termination of primary afferent cardiovascular fibres. Kimmel (1941), using the pyridine silver technique, traced the central course of sensory fibres in the vagus and glossopharyngeal nerves in rabbit fetuses and in rabbits of all ages. He reported that vagal afferent fibres were observed passing to the N. of the tractus solitarius, the N. intercalatus, the N. eminentiae medialis and the N. of Roller. Glossopharyngeal fibres were observed to the N. of the tractus solitarius, the N. intercalatus and the N. praepositus hypoglossi. Fibres to the N. of the tractus solitarius have also been observed in Marchi stained sections of the medulla of the cat after intracranial section of vagal and glossopharyngeal rootlets (Ingram & Dawkins, 1945). However,

fibres were not observed to the other structures implicated by Kimmel. Using the Nauta technique in the rat, Torvik (1956) traced degenerating glossopharyngeal and vagal afferent fibres to the N., of the tractus solitarius, to the rostral hypoglossal N. to the dorsal reticular formation and to the caudal trigeminal N. Using cats, Cottle (1964) also traced degenerating primary afferent fibres with the Nauta technique after section of the glossopharyngeal and vagus nerves intracranially and after selective section of the rostral rootlets of these nerves. She reported that glossopharyngeal fibres projected to the intermediate and rostral portions of the N. of the tractus solitarius, and vagal fibres projected to the intermediate and caudal portions of this nucleus. However, in agreement with the work of Ingram & Dawkins (1945) primary afferent fibres were not observed projecting to other nuclei.

Recording of single unit activity exhibiting spontaneous firing in phase with the heart beat was first used by Smith & Pearce (1961) in the cat to identify medullary structures involved in control of the cardiovascular system. They observed such units only in the region of the N. of the tractus solitarius. These results were subsequently confirmed in the cat by Hellner & von Baumgarten (1961), Salmoiraghi (1962), Middleton & Woolsey (1965) and Seller & Illert (1969), and in the dog by Fussey, Kidd & Whitwam (1967) and Koepchen, Langhorst, Seller, Polster & Wagner

(1967). As single unit activity in phase with the heart beat has been observed only at sites within the N. of the tractus solitarius it seems reasonable to conclude that the N. of the tractus solitarius is the primary site of termination of cardiovascular afferent fibres. This evidence strongly supports the anatomical data of Ingram & Dawkins (1945) and of Cottle (1964) identifying the N. of the tractus solitarius as the only site of termination in the medulla of primary afferent cardiovascular fibres in the IX and X cranial nerves.

Attempts have also been made to determine the central sites of termination of these afferent fibres using the evoked potential technique. In the early application of this technique evoked potentials were recorded in the region of the obex in the medulla during electrical stimulation of the cervical vagus in the cat (Harrison & Bruesch, 1945; Anderson & Berry, 1956) and in the rabbit (Lam & Tyler, 1952). However, these findings are of limited usefulness as histological localization of the sites of recording was not obtained and therefore reliable conclusions cannot be made of the specific medullary structures involved. With the development of histological methods of marking the sites of electrode tips (Hess, 1932, Rayport, 1957) the precise identification of the structures involved became possible. Urabe & Tsubokawa (1960) made small electrolytic lesions at sites from which evoked potentials were recorded in the medulla during

electrical stimulation of the ipsilateral cervical vagus in the cat. Sites marked were observed in the N. of the tractus solitarius, the triangular N. of the vestibular nerve, the N. of the spinal tract of the trigeminal nerve and the N. ambiguus. Unlike the smooth shapes of the responses recorded from most structures, those from the N. ambiguus had a notch in the rising phase. In addition, double stimulation reduced the second deflection in the response recorded from the N. ambiguus (cfr. Eccles, Brock & Coombs, 1953). Therefore the authors concluded that the responses obtained in the vicinity of the solitary tract, the trigeminal N. and the triangular N. of the vestibular nerve were due to orthodromic conduction and those in the N. ambiguus were due to antidromic conduction. Porter (1963), also using cats, recorded evoked activity from single units at histologically verified sites in the N. of the tractus solitarius and in the dorsal N. of the vagus during electrical stimulation of the ipsilateral vagus nerve. As the majority of responses had variable latencies and did not follow stimuli above 50 Hz Porter suggested that the cells from which recordings were made were being synaptically activated by stimulated afferent fibres. Calaresu & Pearce (1965) recorded evoked potentials in the vagus nerve during electrical stimulation of the N. of the tractus solitarius in the cat and concluded that cardiovascular afferent fibres in that nerve projected to the N. of the tractus solitarius. The results obtained using the evoked potential technique are in

agreement with those of anatomical and single unit studies: that the N. of the tractus solitarius receives primary afferent cardiovascular fibres.

The evoked potential technique has also been used to determine the central sites of termination of cardiovascular afferent fibres in other nerves. It has been demonstrated that electrical stimulation of the carotid sinus nerve in the cat elicited firing of units in the medial portion of the N. of the tractus solitarius and that destruction of this area abolished both the reflex decrease in arterial pressure and the vagally mediated bradycardia produced by distension of the carotid sinus (Humphrey, 1967). The termination in the N. of the tractus solitarius of primary afferent cardiovascular fibres from the carotid sinus nerve has been confirmed by others using the evoked potential technique (Crill & Reis, 1968; Sampson & Biscoe, 1968; Miura & Reis, 1969; Seller & Illert, 1969). However, in contrast to the evidence in studies involving the vagi, experiments using the evoked potential technique involving the carotid sinus nerve have shown that fibres of this nerve project to more structures than just the N. of the tractus solitarius. Humphrey (1967) recorded evoked potentials from an area of the medial reticular formation corresponding to the location of the N. paramedium reticularis. Because the evoked potentials recorded in this nucleus were either of long or short latency and because their amplitude could be changed by stimulation of the nucleus of the tractus solitarius it was

suggested that carotid sinus nerve fibres relayed to the medial reticular formation either directly or through the N. of the tractus solitarius. These findings of long and short latency activity in the N. paramedium reticularis during electrical stimulation of the carotid sinus nerve have been confirmed in the cat by Miura & Reis (1969). These investigators also observed evoked potentials in the N. medullae oblongatae centralis, and Crill & Reis (1968) recorded compound action potentials in the carotid sinus nerve of the cat during electrical stimulation of four discrete structures: the N. of the tractus solitarius, the N. paramedium reticularis, the N. medullae oblongatae centralis and the N. gigantocellularis.

Biscoe & Sampson (1970 a, b) stimulated the carotid sinus, glossopharyngeal, aortic and superior laryngeal nerves in the cat and recorded negative field potentials from the N. of the tractus solitarius and from the lateral reticular formation, positive field potentials from the N. medullae oblongatae centralis and from the N. gigantocellularis and evoked single unit activity in the N. gigantocellularis and the N. parvocellularis.

It may be concluded that the evoked potential technique has demonstrated primary afferent cardiovascular fibres to five medullary nuclei: the N. of the tractus solitarius, the N. paramedium reticularis, the N. medullae oblongatae centralis, the N. gigantocellularis, and the N. parvocellularis. The

conclusion, supported mainly by Reis and co-workers, that the N. paramedium reticularis receives fibres directly and indirectly from the carotid sinus nerve has been challenged by Spyer & Wolstencroft (1971) who identified 88 paramedium reticular neurons in the cat by antidromic and orthodromic activation during electrical stimulation of the ipsilateral cerebellar peduncle and did not observe any of these neurons to fire during electrical stimulation of the carotid sinus nerve. They did, however, record field potentials in the vicinity of the N. paramedium reticularis with electrical stimulation of the carotid sinus and hypoglossal nerves, with a larger response during stimulation of the hypoglossal nerve. They concluded that earlier observations of evoked potentials during stimulation of the carotid sinus nerve (e.g. Miura & Reis, 1968, 1969) might have resulted from current spread to the adjacent hypoglossal nerve or from the recording electrode being in a position just lateral to the N. paramedium reticularis. However, Homma, Miura & Reis (1970) have recorded orthodromically evoked action potentials intracellularly from five neurons in the N. paramedium reticularis in the cat during stimulation of the carotid sinus nerve. As the minimum latency was 0.7 msec they concluded that the N. paramedium reticularis receives a monosynaptic input from the carotid sinus nerve. The discrepancy between the results of Spyer & Wolstencroft and the others may be explained on the basis of

two functionally distinct types of neurons in the N. paramedium reticularis, one receiving an input from the carotid sinus nerve and the other receiving an input from or projecting to the cerebellum and receiving no input from the carotid sinus nerve. Evidence for the existence of two populations of neurons in the N. paramedium reticularis has been presented by Calaresu & Thomas (1971) and by Miura & Reis (1971).

A critical analysis of the literature reviewed is necessary before conclusions can be drawn regarding which structures are most likely to be involved in the central control of the cardiovascular system. The pyridine silver technique used by Kimmel (1941) and the Marchi technique used by Ingram & Dawkins (1945) do not yield reliable information due to unselective staining of degenerating fibres. The Nauta technique applied by Torvik (1956) and by Cottle (1964) led to more reliable results as degenerating fibres are stained more selectively than by the earlier techniques. The observations of Torvik and Cottle do not agree except that both demonstrated degenerating fibres to the N. of the tractus solitarius.

Because of the consistency of the findings that phasic single unit activity synchronous with the heart beat has been recorded only from the N. of the tractus solitarius (Smith & Pearce, 1961; Hellner & von Baumgarten, 1961; Salmoiraghi, 1962; Middleton & Woolsey, 1965; Fussey, et al., 1967; Koepchen, et al., 1967; Sellar & Illert, 1969) and because the electro-

physiological evidence was combined with histological verification of the sites of recording it can be reliably concluded that cardiovascular afferent fibres firing in phase with the heart beat terminate in the N. of the tractus solitarius.

The technique of recording of evoked potentials combined with histological localization of the central sites of stimulation and recording provides the most selective means of determining the central sites of termination of specific primary afferent cardiovascular fibres. The most consistent finding obtained with this technique is the demonstration of evoked activity in the N. of the tractus solitarius (Humphrey, 1967; Crill & Reis, 1968; Sampson & Biscoe, 1968; Miura & Reis, 1969; Seller & Illert, 1969; Biscoe & Sampson, 1970 a, b; Homma, Miura & Reis, 1970). Other structures which have been implicated by the evoked potential technique include the N. paramedium reticularis, the N. medullae oblongatae centralis, the N. gigantocellularis, the N. cuneatus, the N. lateralis reticularis and the N. parvocellularis.

To conclude, in view of the implication of the N. of the tractus solitarius as a primary cell station based on both the anatomical and the electrophysiological evidence summarized in Table 1, it appears that this nucleus receives terminals from cardiovascular primary afferent fibres in the carotid sinus, the aortic depressor, the superior laryngeal and the vagus nerves and that the N. paramedium reticularis, the N. medullae oblongatae centralis, the N. gigantocellularis, the N. cuneatus, the N. lateralis reticularis and the N. parvocellularis may also receive primary afferent terminals.

TABLE 1: SUMMARY OF THE LITERATURE ON CENTRAL SITES OF TERMINATION OF PRIMARY AFFERENT
CARDIOVASCULAR FIBRES

Authors and Year	Species	TECHNIQUE	STRUCTURES IMPLICATED
Kimmel, 1941	Rabbit	Silver pyridine staining of central fibres of IX and X nerves	N. of the tractus solitarius, N. intercalatus, N. of Roller, N. eminentiae medialis, N. praepositus hypoglossi
Ingram & Dawkins, 1945	Cat	Marchi staining of degenerating fibres of IX and X nerves	N. of the tractus solitarius
Torvik, 1956	Rat	Nauta staining of degenerating fibres of IX and X nerves	N. of the tractus solitarius, rostral XII N., caudal trigeminal N.

Cottle, 1964	Cat	Nauta staining of degenerating fibres of IX and X nerves	N. of the tractus solitarius
Smith & Pearce, 1961	Cat	Single unit recording from medulla	N. of the tractus solitarius
Urabe & Tsubokawa, 1960	Cat	Electrical stimulation of the vagus; recording from ipsilateral medulla	N. of the tractus solitarius, trigeminal N, triganglular N. of the vestibular nerve
Porter, 1963	Cat	Electrical stimulation of X; recording from medulla	N. of the tractus solitarius, dorsal N. of the vagus
Humphrey, 1967	Cat	Electrical stimulation of carotid sinus nerve; recording from medulla	N. of the tractus solitarius, N. paramedial reticularis

Sampson & Biscoe, 1968	Cat	Electrical stimulation of carotid sinus nerve; recording from medulla	N. of the tractus solitarius
Crill & Reis, 1968	Cat	Electrical stimulation of medulla; recording from carotid sinus and aortic depressor nerves	N. of the tractus solitarius, N. paramedium reticularis, N. medullae oblongatae centralis, N. gigantocellularis, N. cuneatus
Miura & Reis, 1969	Cat	Electrical stimulation of carotid sinus nerve; recording from medulla	N. of the tractus solitarius, N. paramedium reticularis, N. medullae oblongatae centralis
Biscoe & Sampson, 1970 a	Cat	Electrical stimulation of carotid sinus, aortic, superior laryngeal and IX nerves; recording field potentials from medulla	N. of the tractus solitarius, N. lateralis reticularis, N. medullae oblongatae centralis, N. gigantocellularis

Biscoe & Sampson, 1970 b	Cat	Electrical stimulation of carotid sinus, aortic, superior laryngeal and IX nerves; recording single unit activity from medulla	N. gigantocellularis, N. parvocellularis
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Homma, Miura & Reis, 1970	Cat	Electrical stimulation of carotid sinus nerve; recording intracellularly from medulla	N. paramedial reticularis
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(ii) Central connections between structures involved
in cardiovascular control

Little information is available on the connecting pathways between the structures known to be involved in cardiovascular reflex arcs in the brain stem. The earliest attempt at determining these connections resulted in the localization of the medullary depressor area in the N. paramedium reticularis and in the finding that a surgical section between this nucleus and the pressor area of the ventrolateral reticular formation abolished the depressor responses to electrical stimulation of either the vagi or the depressor area (Pórszász, Barankay, Szolcsányi, Gibiszer-Pórszász & Madarász, 1962). The conclusion of this work was that vagodepressor afferent fibres relay to the N. paramedium reticularis which then provides an inhibitory input to the vasoconstrictor centre in the ventrolateral reticular formation. This hypothesis is a significant contribution to the understanding of the central control of the cardiovascular system as it is the first attempt at a general scheme for the functional organization of the discrete structures involved. However, the conclusion that paramedian reticular neurons project to the vasopressor area in the ventrolateral reticular formation based on the evidence that a lesion between the two areas abolishes the depressor response to electrical stimulation of the N. paramedium reticularis is open to criticism because an alternate hypothesis

can account for the results equally well, i.e. that fibres from the N. paramedium reticularis pass ventrolaterally in the medulla to descend in the ventrolateral spinal cord instead of terminating in the medulla. Evidence supporting this alternative hypothesis is that of Illert & Seller (1969) and Illert & Gabriel (1970) who determined the effects on arterial pressure of small lesions in the cervical spinal cord and of electrical stimulation of discrete sites in the cervical spinal cord of the cat and demonstrated that inhibitory pathways to the intermediolateral nucleus are found in the ventrolateral columns of the spinal cord.

The pathways connecting cardiovascular structures has also been investigated by the use of the Nauta technique. Degenerating fibres have been traced by this method from the N. of the tractus solitarius in the cat to the dorsal and lateral reticular formation, ipsilaterally to the N. ambiguus, the dorsal N. of the vagus, the retrofacial N. and the dorsal tegmental N., and bilaterally to the N. intercalatus and the N. praepositus hypoglossi (Morest, 1967). Anatomical pathways from a cardio regulatory area of the posterior hypothalamus to the medulla have also been demonstrated using this technique. Smith (1965) has reported that electrical stimulation of a region in the posterior hypothalamus of the cat induces arterial hypertension and cardioacceleration, and that after destruction of this area axonal degeneration can be traced to the inferior olivary N. and the N. intercalatus in the medulla,

and to the ILN in the spinal cord. In view of the close proximity of the N. intercalatus to the dorsal N. of the vagus and to the N. of the tractus solitarius Smith suggested that the N. intercalatus may be part of a descending hypothalamic pathway which is inhibitory to cardioinhibitory neurons in the medulla. It was later demonstrated that electrical stimulation of the N. intercalatus in the cat elicited cardioacceleration mediated partially by a vagal mechanism (Calaresu & Henry, 1970). Further evidence suggesting that the N. intercalatus may be involved in a descending sympathetic pathway is that evoked activity has been recorded simultaneously from the N. intercalatus and from the inferior cardiac nerve in the dog during cardioacceleration elicited by electrical stimulation of the posterior hypothalamus (Alanis, Mascher & Miyamoto, 1966).

In summary, little information is available on the connecting pathways between structures in the central nervous system involved in control of the cardiovascular system. The few structures implicated by investigations of this type include the N. intercalatus, the inferior olivary N, the dorsal N. of the vagus, the N. praepositus hypoglossi, the N. ambiguus and the N. paramedium reticularis. The literature of this section is summarized in Table 2.

Alanis, et al., 1966	Dog	Electrical stimulation of posterior hypothalamus; recording from medulla	N. intercalatus
Morest, 1967	Cat	Lesion in N. of the tractus solitarius; Nauta method	dorsal N. of the vagus, N. intercalatus, N. praepositus hypo- glossi, N. ambiguus, dorsal and lateral reticular formation.

TABLE 2: SUMMARY OF THE LITERATURE ON CENTRAL CONNECTIONS BETWEEN STRUCTURES INVOLVED IN
CARDIOVASCULAR CONTROL

Author and Year	Species	Methods Employed	Structures Implicated
Pórszász, et al., 1962	Cat	Localization of depressor area by electrical stimu- lation; surgical section between depressor and pressor areas.	N. paramedial reticularis.
Smith, 1965	Cat, dog, and rat	Lesion in posterior hypothalamus; Nauta method	N. intercalatus, inferior olivary N.

(iii) Sites of origin of efferent parasympathetic
cardioregulatory fibres from the medulla

After a review of the literature regarding the central sites of termination of afferent activity from the cardiovascular system and the pathways connecting brain stem structures involved in cardiovascular control, this review will continue with a survey of the literature on the sites of origin of the activity from the brain stem going to regulate the cardiovascular system. This section will discuss the parasympathetic output; the next section will discuss the sympathetic output.

The sites of origin of vagal cardioinhibitory neurons are not known. However, it is likely that the structures giving rise to these fibres are among those implicated in studies aimed at determining the central sites of origin of general vagal efferent fibres. Some evidence regarding the sites of origin of vagal efferent fibres has been obtained from anatomical studies. Section of the medullary rootlets of the vagus and accessory nerves in the rabbit has been shown to produce retrograde degeneration in the dorsal N. of the vagus (Molhant, 1910). Section of the superior cardiac branches of the vagus in the same study produced a small diffuse amount of retrograde

degeneration in well defined areas within this nucleus. Getz & Sirnes (1949) studied retrograde cell changes in the dorsal N. of the rabbit following section of the vagus nerve at four different levels and concluded that the functional representation within the nucleus from the rostral to the caudal end was: lungs and possibly bronchi, abdominal organs, heart and oesophagus, trachea and possibly bronchi. They also showed that when the vagus nerve was cut proximal to the superior laryngeal nerve no normal cells were found within the nucleus, indicating that the dorsal N. is connected exclusively with motor fibres of the vagus nerve. Szentágothai (1952) placed small electrolytic lesions in different parts of the brain stem of the cat and looked for signs of degeneration in peripheral vagal fibres. After placing small lesions in the dorsal N. of the vagus and after ablating the whole ala cinerea he failed to observe any degenerated fibres in the peripheral vagus. After lesions placed in the N. ambiguus, however, he detected signs of degeneration in the cardiac and in the pulmonary branches of the vagus and concluded that pre-ganglionic fibres for ganglion cells of the oesophagus, heart and lungs are localized in the N. ambiguus, especially from the dorso-lateral parts and exclusively from the oral half. Mitchell & Warwick (1955) studied retrograde degeneration in the dorsal N. of the vagus of rhesus monkeys following section of the vagus at different levels and found chromatolytic changes in this nucleus following section of the vagus at all levels. Nishi

(1962) described chromatolytic changes in 70% of the cells of the dorsal N. of the vagus of the mouse following section of the cervical vagus. The evidence from these anatomical studies strongly suggests that the dorsal N. of the vagus is the site of origin of vagal cardioinhibitory fibres. The only contrary evidence to this suggestion is that of Szentágothai who found that the N. ambiguus and not the dorsal N. of the vagus gives rise to vagal cardioinhibitory fibres. The difference between his findings and those of the other investigators may be accounted for on the basis of a species difference as his experiments were done in the cat. Further evidence which supports this possible explanation is the failure to elicit changes in heart rate with electrical stimulation of the dorsal N. of the vagus in the cat (Calaresu & Pearce, 1965; Gunn, Sevelius, Puiggari & Myers, 1968) while stimulation of this nucleus produces a decrease in heart rate in the sheep (Amoroso, Bell & Rosenberg, 1954), the dog (Gunn et al., 1968), the pig (Gootman, Gootman, Buckley, Cohen, Levine & Spielberg, 1972) and the monkey (Kuo, Chai, Lee, Liu & Lim, 1970).

The evoked potential technique has also been used to identify the structures giving rise to vagal efferent fibres. Anderson & Berry (1956) recorded evoked potentials in the medulla of the cat during electrical stimulation of the vagus nerves and claimed they recorded antidromic potentials in the dorsal N. of the vagus and in the N. ambiguus but their sites of recording were not

histologically localized. Porter (1963) recorded from histologically verified sites in the vicinity of the N. ambiguus in the cat during electrical stimulation of the vagus nerve and observed 108 units which had short, unvarying latencies which could be activated by stimulation at 200 - 300 Hz and suggested that these were antidromic responses. In addition, intracellular records in nine of these units demonstrated antidromic spikes. These results were confirmed by Urabe & Tsubokawa (1960). Finally, Gunn et al., (1968) observed that electrical stimulation of the N. ambiguus in the dog and the cat, and of the dorsal N. of the vagus in the dog, consistently elicited evoked potentials in the cervical vagus, but no attempts was made to determine whether the activity was being recorded antidromically or orthodromically.

In summary, the sites of origin of vagal efferent cardio-inhibitory fibres from the medulla are not known. The structures involved may, however, be included among those identified as giving rise to fibres in the vagi. The consistency of the implication of the dorsal N. of the vagus in the anatomical studies cited suggests that this structure gives rise to efferent vagal fibres (cfr. Table 3). The electrophysiological evidence of Anderson & Berry (1956) also implicates this nucleus as well as the N. ambiguus, but they were recording with large electrodes and their localization of the sites of recording was poor. The results of Urabe & Tsubokawa (1960) and of Porter (1963) suggesting the N. ambiguus as the exclusive site of origin of vagal

TABLE 3: SUMMARY OF THE LITERATURE ON THE SITES OF ORIGIN OF EFFERENT CARDIOVASCULAR FIBRES
IN THE VAGUS

Author and Year	Species	Methods Employed	Structures Implicated
Molhant, 1910	Rabbit	Section of vagi; retrograde degener- ation	dorsal N. of the vagus, N. ambiguus
Getz & Sirnes, 1949	Rabbit	Section of vagi; retrograde degener- ation	dorsal N. of the vagus
Szentágothai, 1952	Cat	Small lesions in medulla; peripheral degeneration	N. ambiguus

Mitchell & Warwick, 1955	Rhesus monkey	Section of vagi; retrograde degener- ation	dorsal N. of the vagus
Anderson & Berry, 1956	Cat	Electrical stimulation of aortic and X nerves; record from medulla	dorsal N. of the vagus; N. ambiguus
Urabe & Tsubokawa, 1960	Cat	Electrical stimulation of vagi; record from medulla	N. ambiguus, N. of the tractus solitarius
Nishi, 1962	Mouse	Section of vagi; retrograde degener- ation	dorsal N. of the vagus
Porter, 1963	Cat	Electrical stimulation of vagus; record anti- dromic intracellular activity	N. ambiguus

efferent fibres in the cat are much more reliable because these investigators identified the characteristic antidromically elicited action potentials exclusively within this nucleus during electrical stimulation of the vagus. It can reliably be concluded, then, that the dorsal N. of the vagus and the N. ambiguus give rise to efferent fibres in the vagi. None of the evidence cited, however, has established a functional significance to the fibres originating in these nuclei and therefore no conclusions can be drawn regarding the sites of origin in the medulla of vagal cardioinhibitory fibres.

- (iv) Sites of origin of efferent sympathetic cardiovascular regulatory fibres from the medulla

There is no direct evidence in the literature describing the medullary nuclei which project directly to sympathetic preganglionic neurons in the spinal cord. However, it is likely that at least some of the structures sending terminals to the ILN are among those which have previously been implicated on the basis of evidence from experiments in which cardiovascular parameters were altered during electrical stimulation of the medulla. Therefore, these experiments will be briefly reviewed in this section.

Electrical stimulation of the medulla has been used as a method of localizing cardiovascular regulatory regions of the medulla since the early work of Miller & Bowman (1916) and of Ranson & Billingsley (1916). However, not until recently, with the introduction of more selective methods of stimulation and the technique of marking the sites of stimulation histologically, have specific medullary structures involved in regulation of the cardiovascular system have been identified. Pórszász et al. (1962) have localized the depressor area in vagotomized cats by selective electrical stimulation of the medulla. They reported that depressor responses were most

often obtained with stimulation of sites in the N. paramedium reticularis, with some sites also in the N. gigantocellularis and the raphé NN.. However, as no rostro-caudal coordinates were given for the sites stimulated and because the N. paramedium reticularis and the N. gigantocellularis fuse imperceptibly (Taber, 1961) it is possible that the sites believed to be in the N. gigantocellularis were located in the rostral N. paramedium reticularis. More recently, Calaresu & Henry (1970) have demonstrated that selective electrical stimulation of the N. intercalatus and of a discrete region in the parahypoglossal area in the cat elicits cardioacceleration mediated partly by sympathetic activation and Calaresu & Thomas (1971) have demonstrated that selective electrical stimulation of the N. paramedium reticularis produced cardiac slowing in chloralose-anaesthetized cats by sympathetic inhibition. Experiments designed to determine the effects of electrical stimulation of the medulla on cardiovascular parameters have thus implicated the N. paramedium reticularis and the N. intercalatus in sympathetic regulation of the cardiovascular system.

Cardioregulatory structures may also be among those which have been demonstrated to influence the level of activity in sympathetic nerves. Alexander (1946) stimulated points in the medullary pressor area in the cat and recorded evoked responses in the cervical sympathetic and inferior cardiac nerves. However, his

sites of stimulation were not identified histologically and the specific medullary nuclei being activated were not identified. Kahn & Mills (1967) stimulated medullary pressor and depressor areas in the cat and observed increases and decreases respectively in splanchnic nerve activity. Calaresu & Henry (1970) recorded evoked activity in the cat from pre- and postganglionic thoracic sympathetic nerves to the heart during electrical stimulation of a region in the medulla including the N. intercalatus, the N. medullae oblongatae centralis, the N. interfascicularis hypoglossi and the N. paramedium reticularis. Gootman & Cohen (1971) recorded evoked responses from the splanchnic nerve of the cat during electrical stimulation of medullary vasomotor regions. Single shocks in the pressor region of the dorsolateral rostral medulla, localized histologically in the N. reticularis parvocellularis, where high frequency stimulation produced an increase in arterial pressure, elicited excitatory responses; single shocks in the depressor area in the ventromedial reticular formation where high frequency stimulation produced a decrease in arterial pressure elicited a decrease in splanchnic nerve activity. Taylor & Gebber (1972) recorded evoked activity in a postganglionic branch of the superior cervical ganglion in the cat during stimulation of vasopressor sites in the medullary periventricular grey, the dorsolateral reticular formation, the N. reticularis ventralis and the N. gigantocellularis.

The medullary structures which have been demonstrated to influence the level of activity in sympathetic nerves include the N. intercalatus, the N. parvocellularis, the N. gigantocellularis, the N. ventralis reticularis, the ventromedial reticular formation, the dorsolateral reticular formation and the periventricular grey. It is possible that some of these structures project monosynaptically to ILN neurons, but definite conclusions cannot be made because the methods employed do not demonstrate whether the structures stimulated project directly or indirectly to spinal sympathetic neurons.

Finally, it has been demonstrated using anatomical techniques, that neurons in the medullary raphé NN. and in the ventrolateral reticular formation likely project directly to spinal sympathetic neurons. Using the fluorescent histochemical method of Carlsson, Falk, Fuxe & Hillarp (1964), Dahlström & Fuxe (1965) demonstrated in the cat and several other species that ILN neurons receive a large number of terminals containing noradrenaline and 5-hydroxytryptamine. As they had previously demonstrated (Dahlström & Fuxe, 1964) that noradrenaline containing neurons could be found in the ventrolateral part of the reticular formation in the caudal medulla and that 5-HT (tryptaminergic) neurons were found in the raphé NN. they attempted to find whether the medullary noradrenergic and 5-HT neurons projected to the spinal cord. After treatment with nialamide, a potent MAO-inhibitor which causes accumulation

of 5-HT in the axons of tryptaminergic neurons, a majority of the 5-HT nerve cells in the raphé NN. of the caudal medulla were observed sending axons to the spinal cord. In addition, total transection of the spinal cord led to degenerative changes in these neurons, particularly a decreased capacity to accumulate 5-HT after MAO inhibition. After transection of the spinal cord the catecholamine neurons in the ventrolateral part of the reticular formation showed a marked increase in their amine counts, again, probably due to the fact that their axons were cut during spinal transection. In addition, catecholamine fibres were traced from the white matter of the spinal cord to the most caudal part of the medulla oblongata and no further. Finally, after transection of the spinal cord a piling-up of noradrenaline and 5-HT was observed just above, but not below, the level of the section, and the capacity of 5-HT terminals and axons to store and synthesize 5-HT after MAO inhibition was observed to disappear approximately five days after the transection. Dahlström, Fuxe, Kernell & Sedvall (1965) have also demonstrated that prolonged stimulation of the medulla in rats pretreated with amine synthesis inhibitors causes a greater reduction of noradrenaline and 5-HT stores than in rats given amine synthesis inhibitors only.

This evidence, combined with that of DeGroat & Ryall (1967) and of Ryall (1967) that local iontophoretic application of 5-HT

and noradrenaline to neurons of the ILN in the cat produced activation and depression, respectively, of these cells, suggests that the 5-HT-containing neurons of the medullary raphé NN. serve to activate spinal sympathetic neurons while the noradrenaline-containing neurons of the ventrolateral reticular formation serve to inhibit the activity of the sympathetic neurons.

In summary, there is no direct evidence in the literature identifying the sites of origin of descending fibres from medullary structures to sympathetic preganglionic neurons in the spinal cord (cfr. Table 4). Two structures have been implicated in the central sympathetic control of the cardiovascular system. These include the N. paramedium reticularis (Pórszász et al., 1962; Calaresu & Thomas, 1971) and the N. intercalatus (Calaresu & Henry, 1970). Other structures which have been demonstrated to influence the level of sympathetic activity include the N. parvocellularis, the ventro-medial reticular formation (Gootman & Cohen, 1971), the dorsolateral reticular formation, the N. reticularis ventralis and the N. gigantocellularis (Taylor & Gebber, 1972). The electrophysiological techniques employed cannot, however, demonstrate whether the structures implicated project directly or indirectly to spinal sympathetic neurons. The Nauta technique has been used to demonstrate a monosynaptic connection between the posterior hypothalamus and the ILN (Smith, 1965), but this technique has not been used to determine the medullary sites of origin of descending fibres to the ILN.

TABLE 4: SUMMARY OF THE LITERATURE ON THE SITES OF ORIGIN OF DESCENDING CARDIOVASCULAR FIBRES
FROM THE MEDULLA TO THE SPINAL CORD

Author and Year	Species	Methods Employed	Structures Implicated
Pórszász et al., 1962	Cat	Electrical stimulation of medulla; record arterial pressure	N. paramedial reticularis, raphé NN., N. gigantocellularis
Dahlström & Fuxe, 1965	Cat	Fluorescence histochemistry	raphé NN., N. lateralis reticularis
Calaresu & Henry, 1970	Cat	Electrical stimulation of medulla; record heart rate; record from sympathetic cardiac nerves	parahypoglossal area including N. intercalatus

Calaresu & Thomas, 1971	Cat	Electrical stimulation of medulla; record heart rate	N. paramedium reticularis
Gootman & Cohen, 1971	Cat	Electrical stimulation of medulla; record from splanchnic nerve	N. parvocellularis, ventro-medial reticular formation
Taylor & Gebber, 1972	Cat	Electrical stimulation of medulla; record from superior cervical ganglion	medial periventricular grey, dorso-lateral reticular formation, N. reticularis ventralis, N. gigantocellularis

Only the histochemical technique used by Dahlström & Fuxe (1965) has identified sites of origin of descending fibres terminating on neurons in the ILN but no functional significance has been attached to these connections.

D. Conclusions and Objectives of the Study

A critical review of the literature pertaining to the medullary structures implicated in the central control of the cardiovascular system has been presented. Several conclusions may be made from this review.

Primary afferent fibres involved in cardiovascular reflex arcs project to specific structures in the medulla. However, apart from the projection from the N. of the tractus solitarius to the N. paramedium reticularis, the secondary cell stations in cardiovascular reflex arcs remain to be determined.

In addition, there is essentially no reliable evidence regarding the intramedullary connections between other cardiovascular structures in the medulla which are involved in descending pathways from supramedullary structures or in complex reflex arcs within the medulla.

Finally, although it appears likely that efferent cardiovascular fibres in the vagi originate in the dorsal N. of the vagus or in the N. ambiguus, there have been no attempts to determine the sites of origin of descending cardiovascular fibres projecting to sympathetic preganglionic neurons in the spinal cord.

In view of the fact that several medullary structures have been implicated in the central control of the cardiovascular

system but that little information is available regarding the connections between these structures and regarding the sites of origin of efferent cardiovascular fibres from the medulla a significant contribution to the understanding of the functional organization of cardiovascular reflex arcs in the medulla would be the identification of the medullary structures on the efferent side of these reflex arcs. With this information connections between the structures on the afferent and efferent sides of the reflex arcs may be investigated and the knowledge of the circuitry of mechanisms of central control may be more complete.

Therefore, the objective of this study was to determine the medullary structures projecting to sympathetic preganglionic cardioacceleratory neurons in the ILN by activating the terminals of the descending fibres as they enter the ILN and exploring the medulla systematically for evoked antidromic responses. As the spinal representation of cardioacceleratory neurons is not known, and as this information was necessary to ensure selective activation of the descending cardio regulatory fibres the two projects described in the INTRODUCTION were completed before the search for efferent cardiovascular structures in the medulla could be undertaken.

METHODS

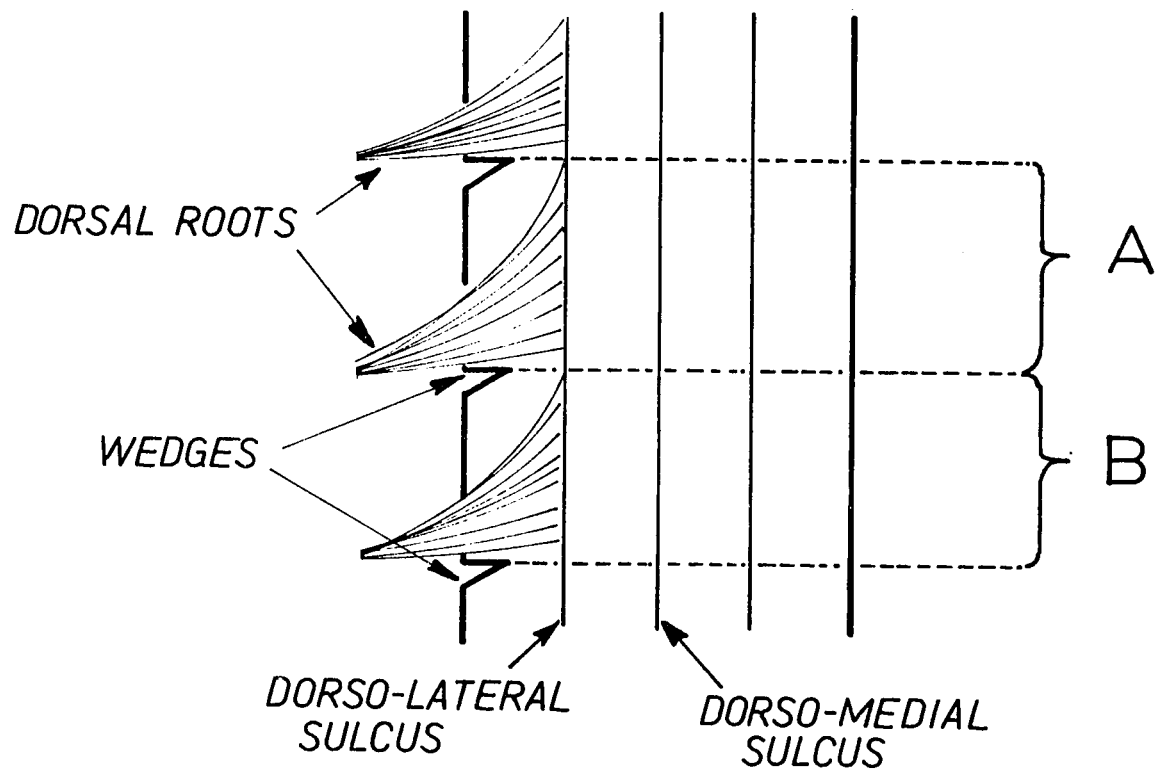
A. Histology of the Intermediolateral Nucleus

(a) General Procedure

Four male and four female adult cats (2.2 to 3.0 kg) were anaesthetized and perfused with 0.9% saline followed by 10% formol-saline. The spinal cords were removed and fixed in 10% formol-saline for at least two weeks. After fixation the meninges were removed and each segment was marked by cutting out a small wedge of tissue from the left side of the cord at the level of entry of the most rostral dorsal root bundles. In this work a segment of the cord includes the tissue between the rostral borders of two successive wedges and is identified by the number of the corresponding dorsal root. Figure 4, a schematic diagram of the locations of wedges of tissue removed from the spinal cord, illustrates this definition of "segment". Each spinal cord, trimmed to include segments C₆ to L₅, was divided into four parts by three transverse cuts perpendicular to the longitudinal axis of the cord at levels corresponding to the rostral borders of three wedges. After embedding in paraffin, serial transverse sections 15 μ m thick were cut and every tenth section was mounted and stained with thionin by the

FIGURE 4

Schematic diagram illustrating the locations of wedges of tissue removed from the spinal cord to mark segmental levels. A and B represent consecutive segments.



method of Clark & Sperry (1945). A total of 9400 sections was studied.

(b) Criteria for Identification of ILN Neurons

The neurons of the ILN are either bipolar or multipolar and are usually pyriform in shape with the long axis two to three times the length of the short axis. Their nuclei are located in a central position and contain darkly stained nucleoli. The scanty cytoplasm contains Nissl substance arranged in packets of irregular size and shape, a characteristic attributed to efferent neurons (Crosby, Humphrey & Lauer, 1962). The appearance of typical ILN neurons is shown in Figures 5 and 6. The distinction between neurons and glial cells is unequivocal because neurons contain nucleoli clearly surrounded by nucleoplasm. Neurons of the ILN are easily differentiated from those in neighboring structures at the lateral and dorsal borders because of intervening white matter. The ventral border is usually distinct as ILN and ventral horn neurons are different in size and are clearly separated. The medial border, however, is occasionally difficult to establish because of the presence of scattered neurons between the ILN and the intermediomedial nucleus; in these cases only those neurons which clearly appeared as part of a group of other ILN cells were included.

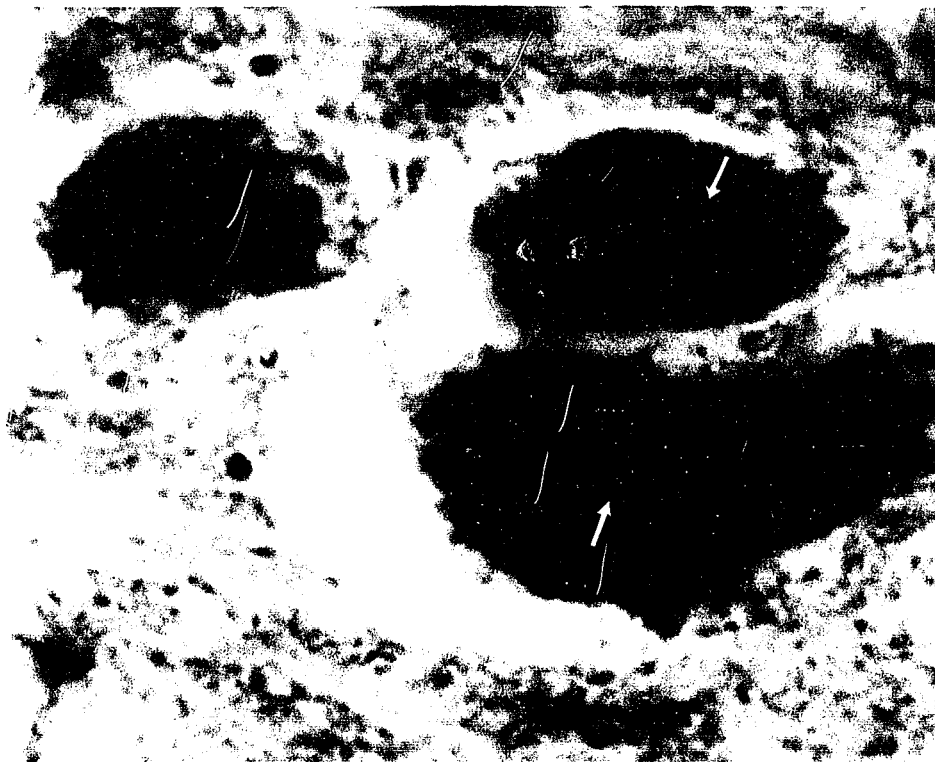
FIGURE 5

Typical appearance of neurons in the ILN
at the level of T₁. Note the character-
istic pyriform shape, the scanty cytoplasm
and the packets of Nissl substance.
Calibration 10μm.



FIGURE 6

A group of three neurons in the ILN at the level of T₉. Arrows point to the nucleoli of the two cells counted. Calibration 10 μ m.



(c) Counts and Distribution of ILN Neurons

In every section the number of neurons in the right and left intermediolateral nuclei was determined by counting nucleoli at a magnification of x 320 using a Carl Zeiss RA microscope. An estimate of the total number of neurons was then obtained by multiplying the counts by ten. The accuracy of this method was assessed by comparing the estimated count with the actual count of nucleoli in a series of 200 consecutive serial sections in one segment of one specimen: the numbers of counted and estimated nucleoli were 2022 and 1980, respectively. The small difference (2%) indicates that the method used provides a good estimate of the actual number of neurons.

To obtain information on the longitudinal distribution of neurons throughout the spinal cord counts were obtained in consecutive divisions, each 2.55 mm in length. This length was chosen because it provided adequate detailed information about the distribution of neurons yet eliminated the wide fluctuations in counts observed from slide to slide. Using a linear shrinkage factor of 15%, derived from a 38% volumetric shrinkage factor demonstrated to occur in tissue after formol fixation, dehydration and paraffin embedding (Baker, 1956), this length of 2.55 mm in fixed tissue corresponds to a length

of 3.0 mm in unfixed tissue. The numbers of cells counted in consecutive 3 mm lengths of unfixed tissue were then plotted against distance as well as against segments of the spinal cord.

(d) Estimates of Neuron Size

To obtain an estimate of the distribution of neuron sizes every tenth section mounted was examined in one specimen from a male cat. The long and short diameters of neurons containing an identifiable nucleolus were measured at a magnification of x 500 using a Leitz filar micrometer eyepiece calibrated against a stage micrometer. The two diameters of each neuron were then multiplied to obtain a quantitative estimate of the area of the cell. A total of 369 neurons in 116 sections was measured. No attempt was made to correct the estimate of neuron sizes for shrinkage because precise measurements of the areas of the neurons were not made.

B. Mapping of Cardioacceleratory Sites in the Spinal Cord

(a) General Procedure

Thirty-two cats were used in this series of experiments. The cats, between 2.3 and 3.8 kg, were anaesthetized with 80 mg/kg of alpha chloralose (British Drug Houses Ltd., Poole, England) in saline, injected intravenously after ethyl chloride and ether induction. Arterial pressure, monitored by a Statham transducer connected to a catheter in the left femoral artery, heart rate, computed by a Grass 7P4 tachograph preamplifier, and respiration, monitored as a change in the temperature of tidal air by a thermistor in the tracheal cannula, were recorded on a Grass 7 polygraph. The heads of the animals were fixed in a Kopf stereotaxic instrument; the ilia and the spinous processes of the seventh cervical and ninth thoracic vertebrae were held rigidly in a Kopf spinal unit. The dorsolateral surface of the spinal cord, from C₇ to T₉, was exposed by laminectomy and the spinal cord was transected at C₇ to eliminate the supraspinal input to sympathetic preganglionic neurons. Warm mineral oil was poured over exposed nervous tissue to prevent drying. The cervical vagi were cut to eliminate changes in heart rate due to changes in the level of activity of vagal cardioinhibitory fibres. To ensure that cardiovascular responses elicited by electrical stimulation of the spinal cord were not due to changes in the level of circulating adrenal catecholamines the adrenal

glands were removed. The mean arterial pressure was maintained at approximately 90 mm Hg by the continuous infusion of nor-adrenaline in physiological saline (60 - 240 μ g/ml) into the left saphenous vein at a rate of infusion which did not exceed 3 ml/kg \cdot hr and was usually less than 0.5 ml/kg \cdot hr. The rectal temperature was maintained between 37 and 38 $^{\circ}$ C by a heating pad controlled by a Yellow Springs 73 temperature controller.

(b) Stimulation of the Spinal Cord

Selective electrical stimulation of the ILN was attempted using stainless steel unipolar electrodes (shaft diameter 175 μ m, tip diameter 50 μ m) insulated with insl-X (insl-X Co., Ossining, New York) to within approximately 150 μ m from the tip. The integrity of the insulation was determined by applying 6 volts d.c. between the electrode and an alligator clip immersed in saline. With the electrode as the cathode, the formation of bubbles at places other than the tip indicated unsatisfactory insulation. The d.c. resistance in saline was between 40 and 60 k Ω . The indifferent electrode was an alligator clip attached to exposed back muscles. The stimulus used, except in selected experiments, was a train of rectangular pulses (6.4 V, 0.2 msec, 20 Hz) delivered for periods up to 60 sec. This voltage was selected because it was found to be adequate for suprathreshold stimulation of the ILN.

C. Recordings from the Medulla During Stimulation of the
Spinal Cord

(a) General Preparation

Twenty-nine cats, between 2.3 and 3.5 kg, were anaesthetized with 80 mg/kg alpha chloralose after ethyl chloride and ether induction. Arterial pressure, heart rate and respiration were monitored, and the animals were fixed rigidly in head and spinal holders; the details of these procedures are described under METHODS, section B.

The floor of the fourth ventricle was exposed by removing the occipital bone and plugging the cut edges of the bone with Horsley's bone wax to stop bleeding. The cerebellum was partially removed by suction. The dorsolateral surface of the spinal cord, from T₁ to T₄, was exposed by laminectomy. Warm mineral oil was poured over exposed nervous tissue to prevent drying. The cervical vagi were cut to ensure that changes in heart rate occurring during the experiment were due to sympathetic mechanisms only.

(b) Stimulation of the Spinal Cord

Selective electrical stimulation of the ILN was attempted using stainless steel unipolar electrodes similar to those described under METHODS, section B, but with tip diameters

of three to ten μm and shaft diameters of approximately 175 μm . The indifferent electrode was an alligator clip attached to exposed back muscles at the caudal end of the exposure. The stimulus used was a train of rectangular pulses of 6 - 10 V (approximately 200 μA), 0.2 msec and 20 Hz. The second thoracic segment was selected for stimulation because it was demonstrated that ILN neurons are most abundant in this segment (see Figure 17) and that sympathetic preganglionic cardioacceleratory neurons are located in this part of the spinal cord (see Figure 20). Stimulation was on the right side only because it was demonstrated that cardioacceleratory neurons are more abundant on the right side than on the left (see Table 6).

During the experiment the tip of the spinal electrode was in the ILN when stimulation elicited an increase in heart rate and in arterial pressure.

(c) Recordings from the Medulla

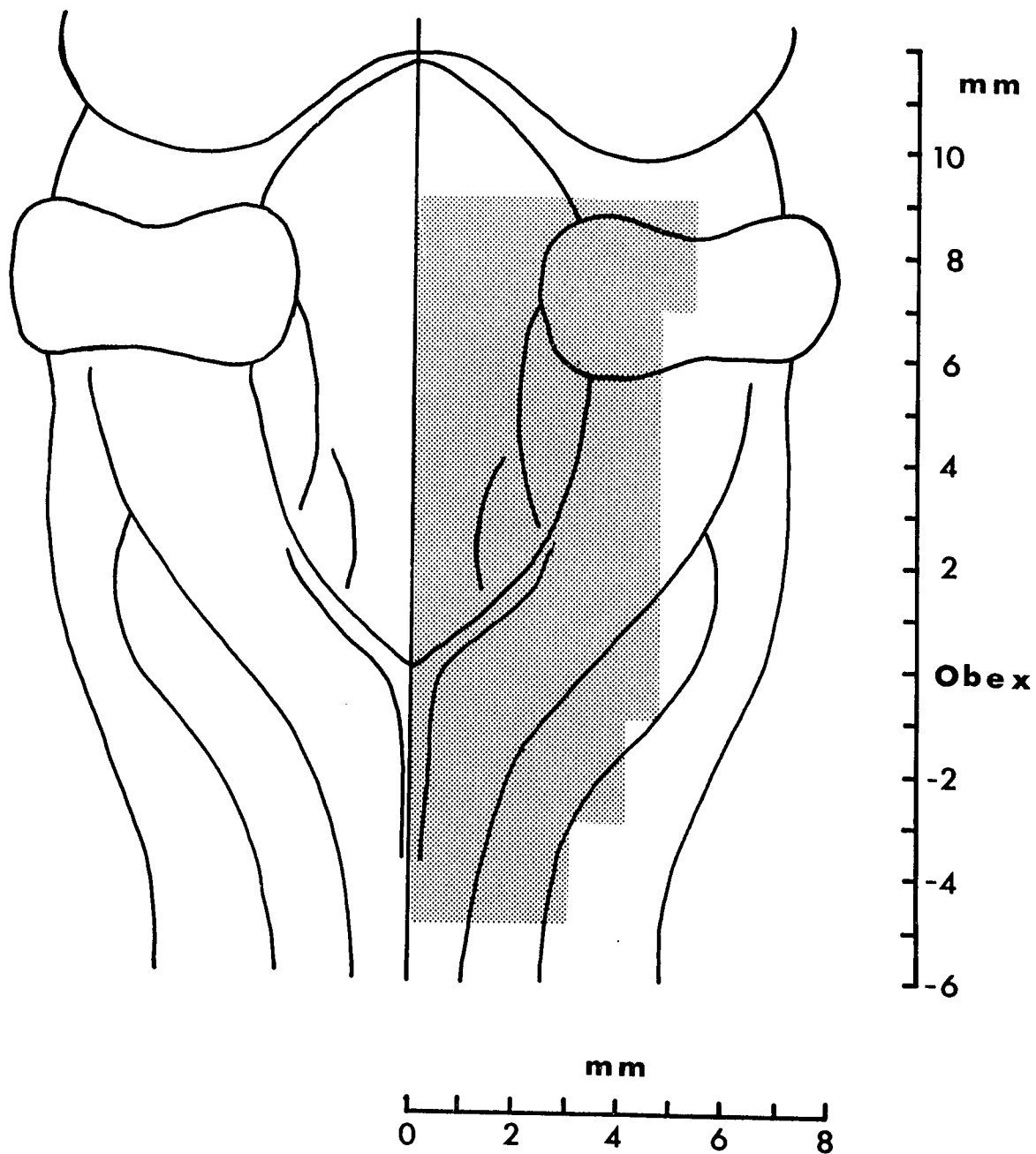
Recording of evoked activity from the medulla was attempted only during electrical stimulation of a site in the ILN from which cardioacceleration could be elicited. Concentric bipolar stainless steel electrodes (Transidyne model SNE-100) with a tip diameter of approximately 50 μm , a tip separation of approximately 500 μm , and a shaft diameter of approximately 300 μm were used. Bipolar rather than monopolar electrodes were

used as it has been demonstrated that when using large electrodes (tip diameter, 180 μ m) evoked responses recorded monopolarly are insufficiently selective to map precise potential fields in the diencephalon whereas evoked responses recorded bipolarly from the same region are more localized (Rudomin, Malliani, Borlone & Zanchetti, 1965). In addition, monopolar electrodes have been used effectively in those regions, such as the spinal cord (Eccles, Fatt, Landgren & Winsbury, 1954; Wall, 1962), which are very close to the site of stimulation but have been less useful when the site of recording is farther away and when the evoked responses are of small amplitude (Rudomin et al., 1965)

The recording electrodes were carried in an electrode holder which allowed movements of 100 μ m in the three orthogonal planes. A region of the medulla extending from the mid line to five mm to the right side and from five mm caudal to nine mm rostral to the obex was systematically explored. Figure 7 illustrates the location of the region explored. The first penetration in any experiment was at a predetermined level rostral or caudal to the obex and in the mid line. Successive penetrations were made in steps of 0.5 mm laterally until the lateral limit of the area to be explored was reached. Then another penetration was begun at a different rostro-caudal level, again beginning in the mid line and working laterally. Stereotaxic coordinates of the point of entry of the electrode into the medulla were determined visually with respect to the obex. The vertical

FIGURE 7

Drawing of dorsal surface of medulla.
Hatched area shows region of medulla
explored.

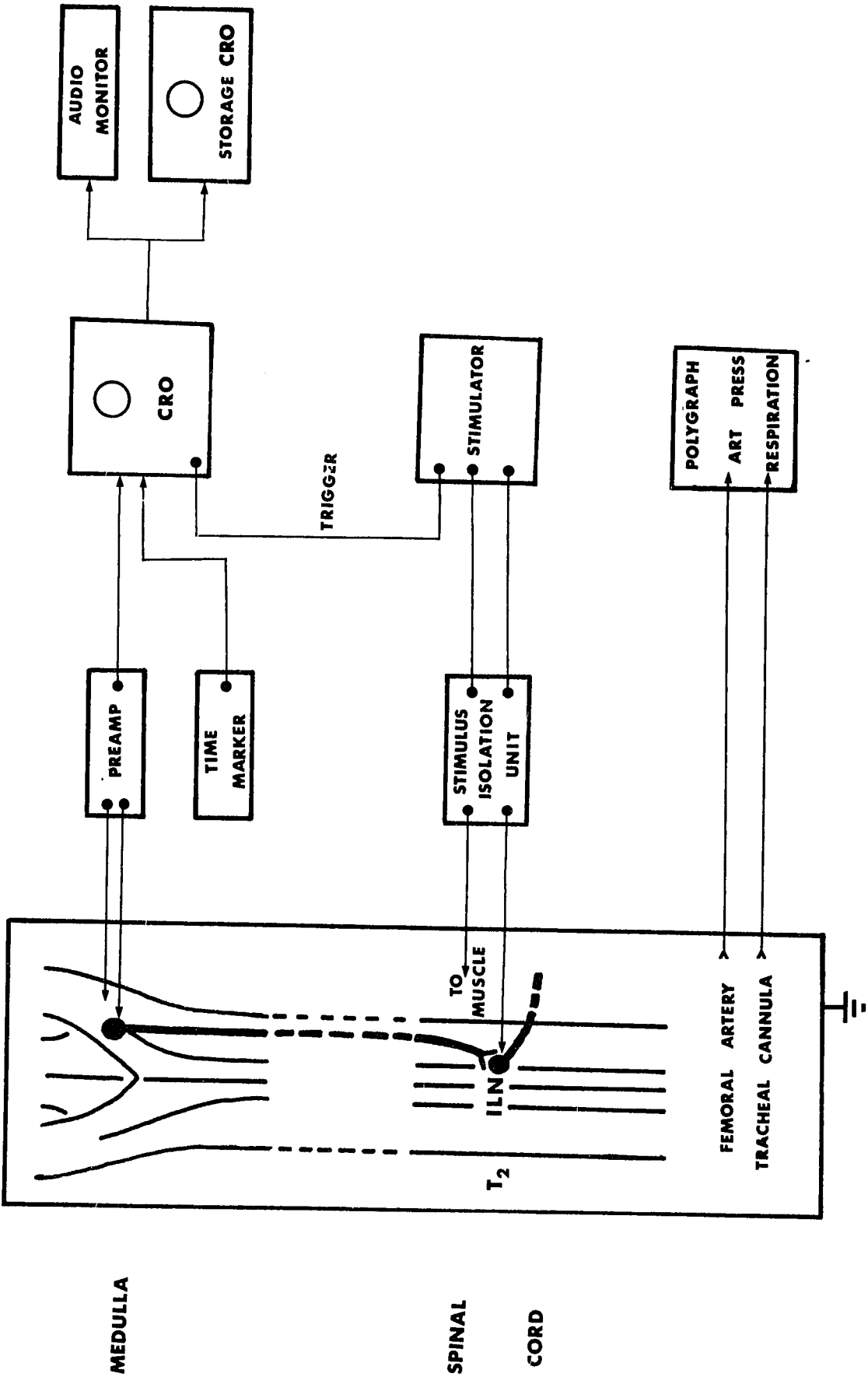


reference was with respect to the floor of the fourth ventricle at the point of entry of the electrode.

While the medulla was being systematically explored for evoked responses the ILN was repetitively stimulated at 0.5 to 0.7 Hz at a stimulus intensity similar to that required to elicit the cardiovascular responses. The recorded activity was amplified by a Grass P14 preamplifier and displayed on the screen of a Tektronix model 565 oscilloscope through a Tektronix model 2A61 differential amplifier. The bandwidth of the recording apparatus was set to include frequencies between 0.1 and 50 kHz. The signal from the 565 oscilloscope was fed to a Tektronix model 564 storage oscilloscope and to a Grass audio amplifying system. A diagram of the experimental arrangement used is shown in Figure 8. Selected records of superimposed sweeps were photographed from the face of the storage oscilloscope by a Tektronix model C-27 oscilloscope camera on Polaroid Land film type 107.

FIGURE 8

Schematic diagram showing experimental
arrangement used for stimulation of the
spinal cord and recording from the medulla.



D. Histological Localization of the Sites of Stimulation and Recording

As accurate anatomical localization of the tips of the stimulating and recording electrodes could not be obtained by relying on stereotaxic coordinates, and to ensure positive identification of the sites of stimulation and recording, histological localization of the positions of the electrode tips was obtained. Iron deposits were produced from the tips of the electrodes by passing a d.c. current of 5 microamps for 30 sec, with the electrode as the anode. At the end of each experiment, the animal was perfused with saline and then with 1% potassium ferrocyanide in 10% formol to stain the deposits. The medulla and spinal cord were then removed and fixed for three to seven days in a solution of two parts of 2% acetic acid in 95% ethyl alcohol and eight parts of 1% potassium ferrocyanide in 10% formalin. At the end of the fixation period, the medulla was washed in running water for one to three hours and the meningeal membranes were removed. Frozen serial sections, 50 μ m thick, were cut and mounted on slides. After drying, the sections were dehydrated in 100% alcohol and taken through a descending alcohol series back to water and stained for 30 minutes in a thionin solution (1% thionin, 0.8% sodium acetate, 0.6% acetic acid, with pH adjusted to 3.7). The slides were then taken

through an ascending alcohol series to xylol and covered, with Gurr's D.P.X. as the mounting medium. Iron deposits appeared as blue spots against the pale blue background. An example of an iron deposit in the medulla is shown in Figure 9.

Because difficulties were encountered in keeping the spinal cord frozen during cutting, after fixation, the cords were taken through an ascending alcohol series to xylol and embedded in paraffin wax. Serial transverse sections, 15 μ m thick, were cut and every tenth section mounted. After drying, the sections were stained with thionin by the same method as above. An example of an iron deposit in the ILN is shown in Figure 10.

FIGURE 9

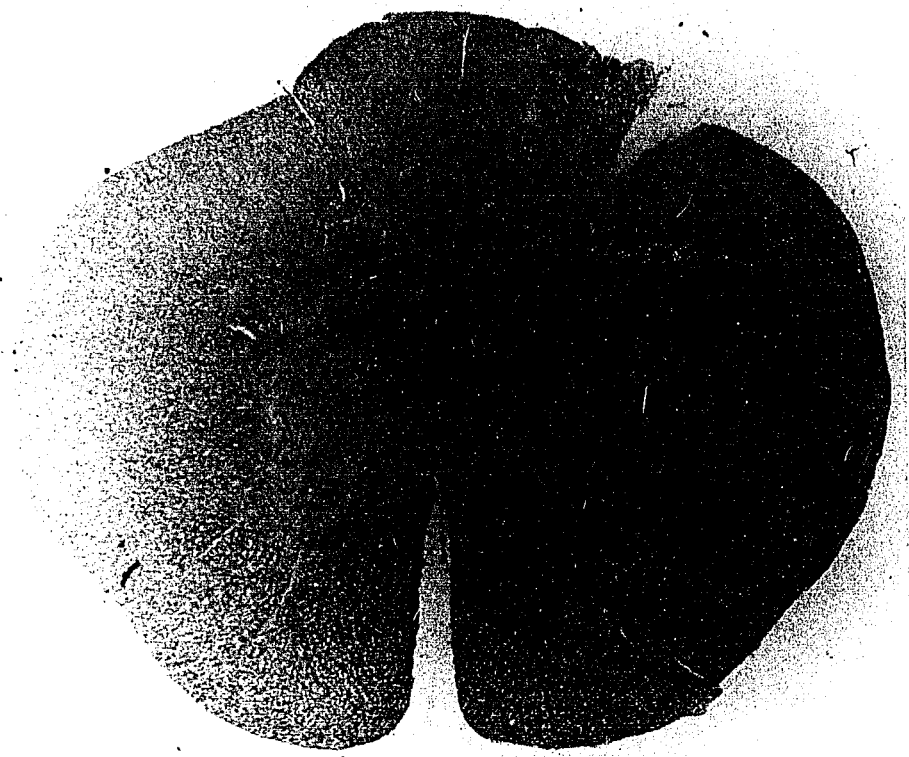
Transverse section of the medulla of a cat showing an iron deposit (at arrow) at a site from which an evoked response was recorded. Thionin and potassium ferrocyanide stains. Calibration 1 mm.



—

FIGURE 10

Transverse section of the thoracic spinal cord of a cat showing an iron deposit at a site of stimulation within the intermediolateral nucleus. Thionin and potassium ferrocyanide stains. Calibration 1 mm.



RESULTS

A. Anatomy of the Intermediolateral Nucleus

(a) Topography of the ILN

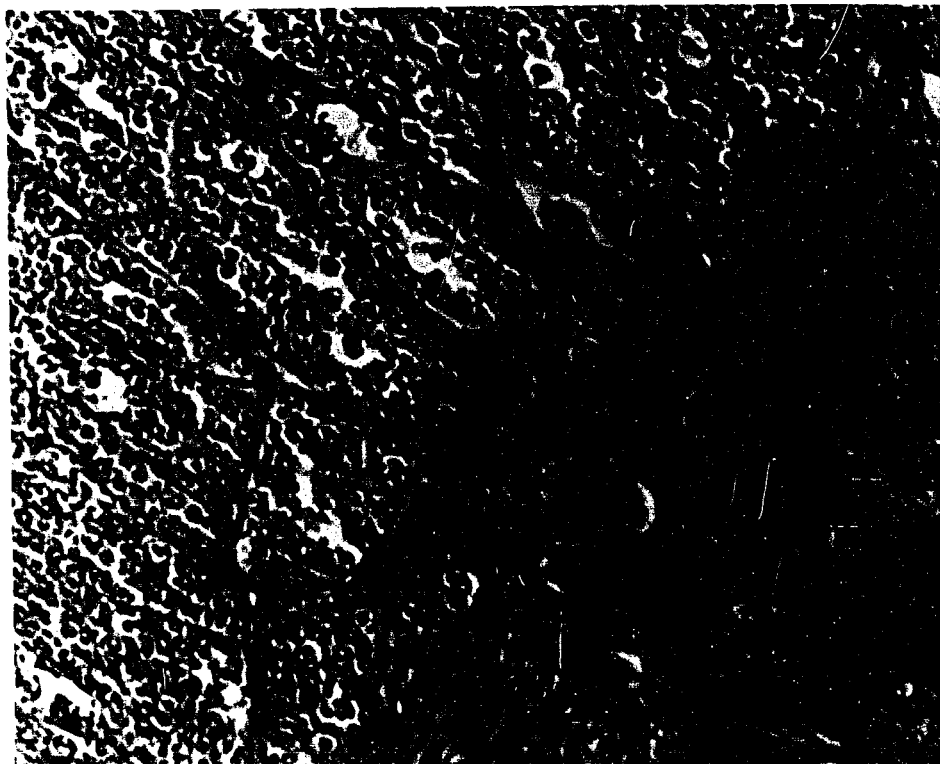
The ILN was readily recognized within the lateral horn in all segments (Figs. 11, 12, 13, 14) except in segments C₈ - T₁ and L₁ - L₂ where the neurons of the ILN were found scattered in finger-like projections of grey matter extending into the white matter. The typical appearance of these projections is shown in Figures 15 and 16. The rostral pole of the ILN was consistently found at the junction of C₈ - T₁ and the caudal pole at the level of L₄.

(b) ILN Neuron Counts

The values of the estimated total neuron counts ranged from 32,790 to 53,340. The mean counts for the right and left sides were not significantly different ($p > 0.6$). However, a statistically significant ($p < 0.02$) difference was demonstrated between the mean neuron counts in the four males ($45,765 \pm 2,556$) and those in the four females ($35,543 \pm 1,411$). Total counts, counts for the left and right sides, sex, body weights and lengths of the ILN (unfixed) of each animal are presented in Table 5.

FIGURE 11

Transverse section of a portion of the spinal cord at the level of T₅ illustrating the characteristic distribution of neurons in the lateral horn; this distribution was observed throughout the extent of the ILN except at the levels of C₈ - T₁ and L₁ - L₂. In this section four nucleoli were counted. Calibration 50 μ m.



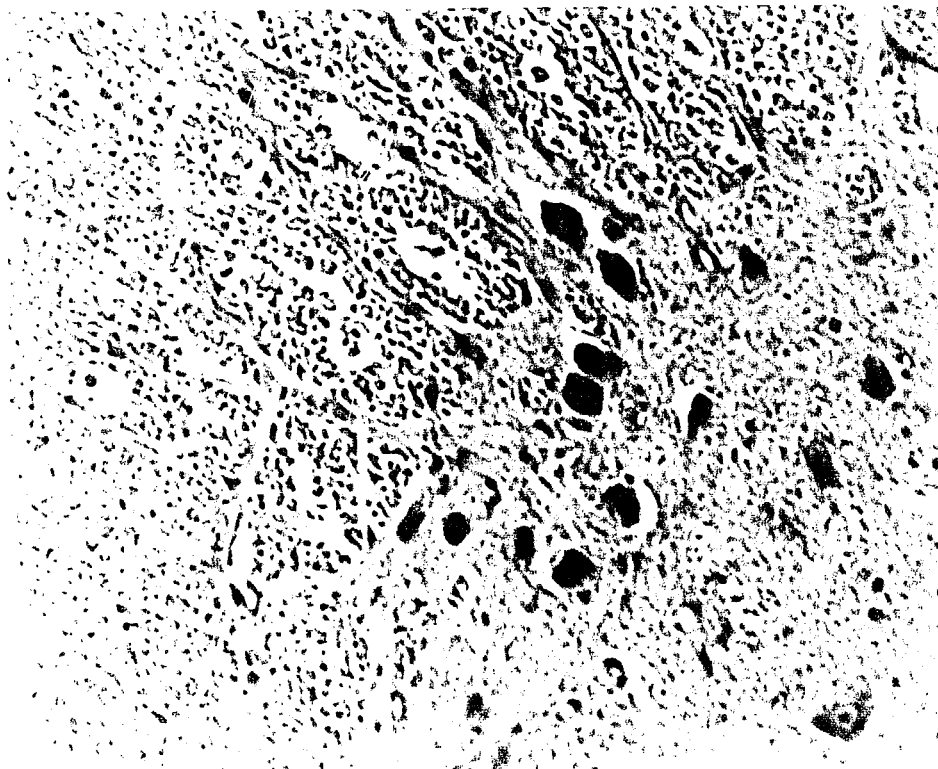
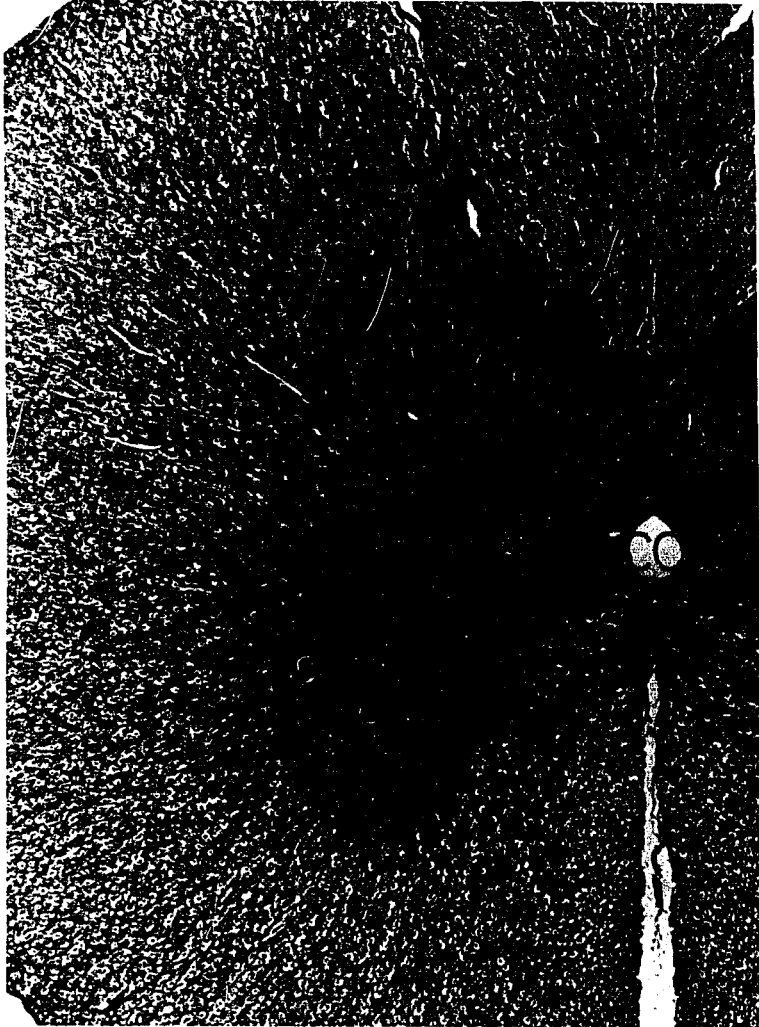


FIGURE 12

Transverse section of the spinal cord at the level of T₅. The neurons demarcated by the line were considered to be in the ILN. CC, central canal; D, dorsal horn of grey matter; V, ventral horn of grey matter. Calibration 0.5 mm.



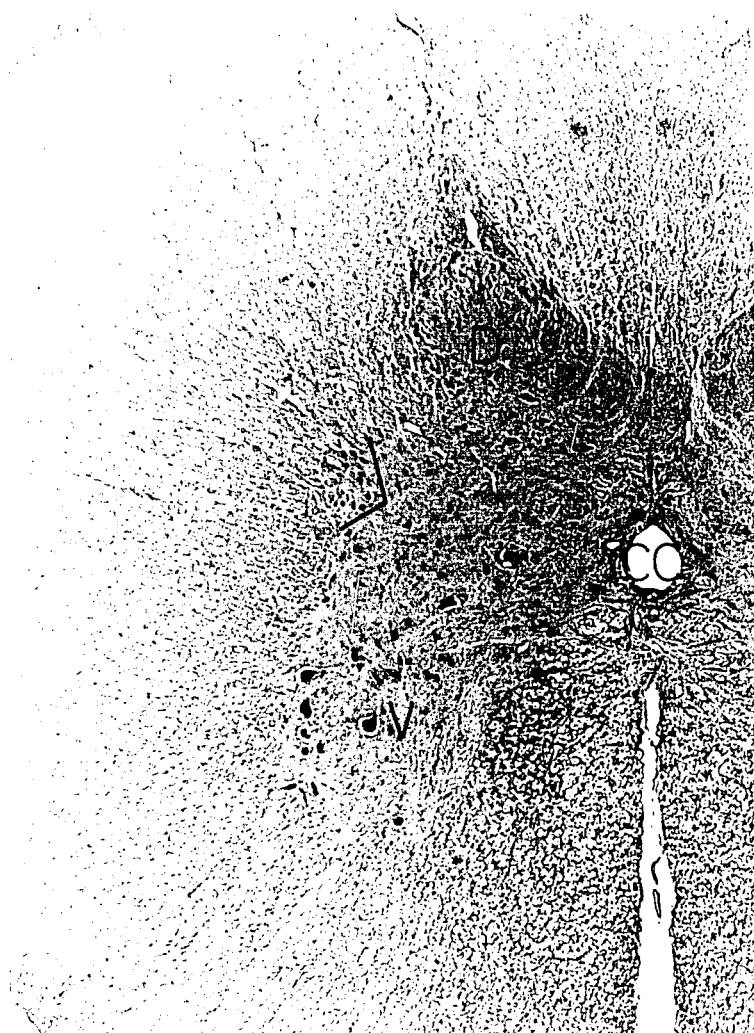
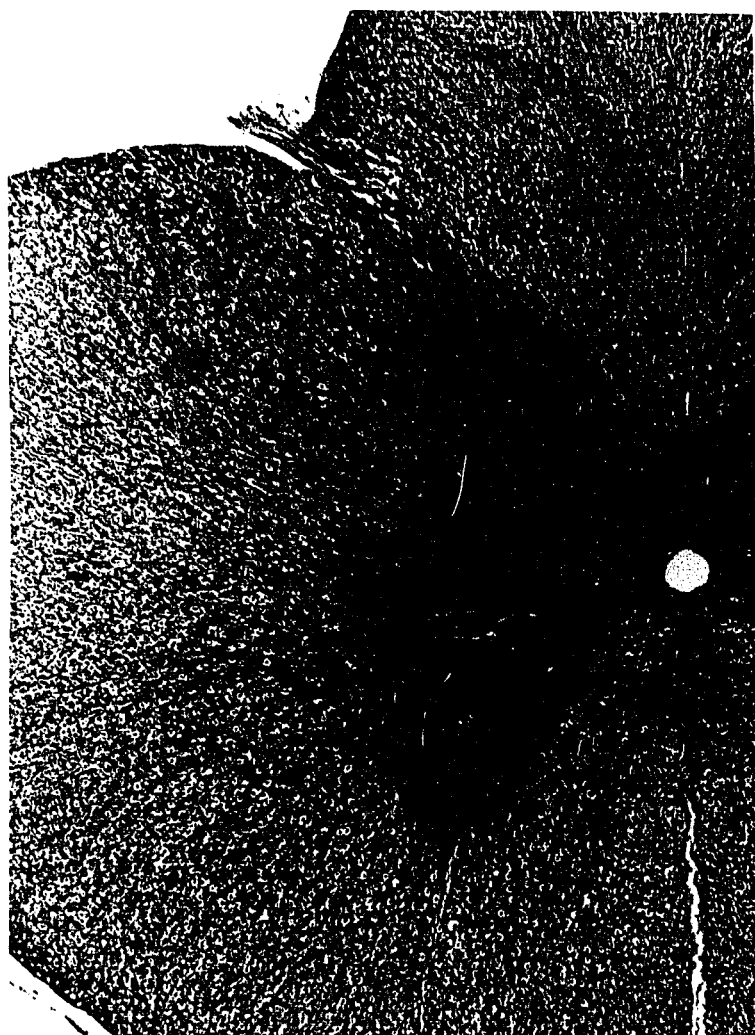


FIGURE 13

Transverse section of the spinal cord at the level of T₁₃. The neurons demarcated by the line were considered to be in the ILN. Calibration 0.5 mm.



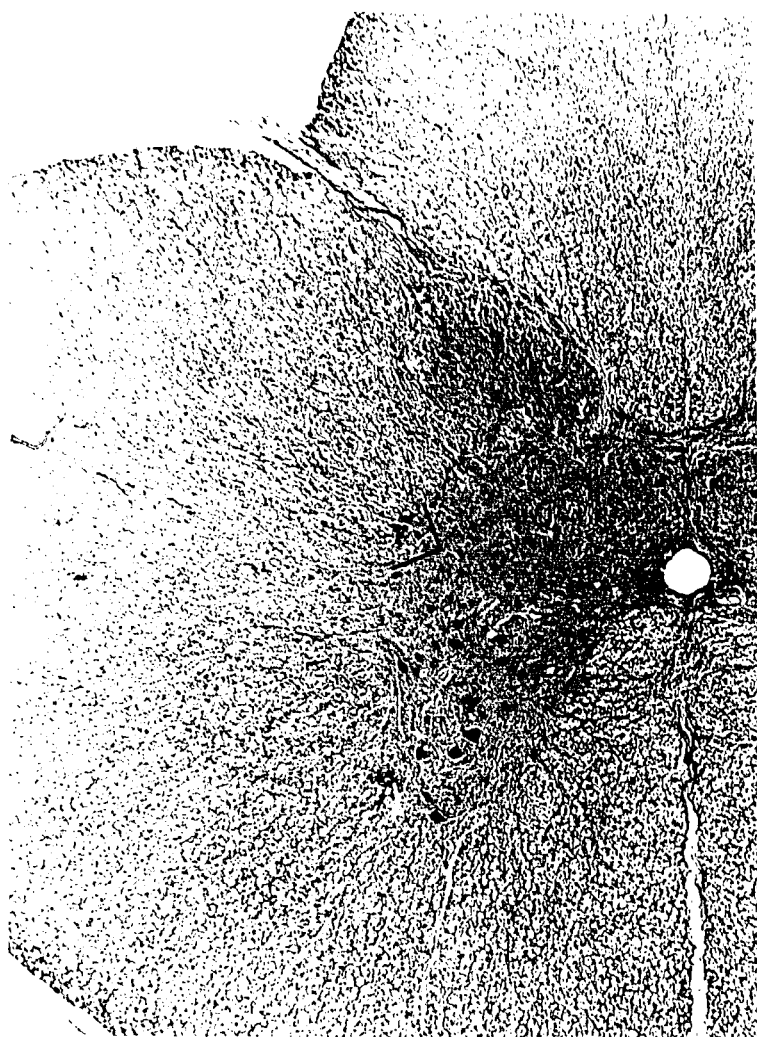


FIGURE 14

Transverse section of the spinal cord at the level of L_4 . The neurons demarcated by the line were considered to be in the ILN. Calibration 0.5 mm.



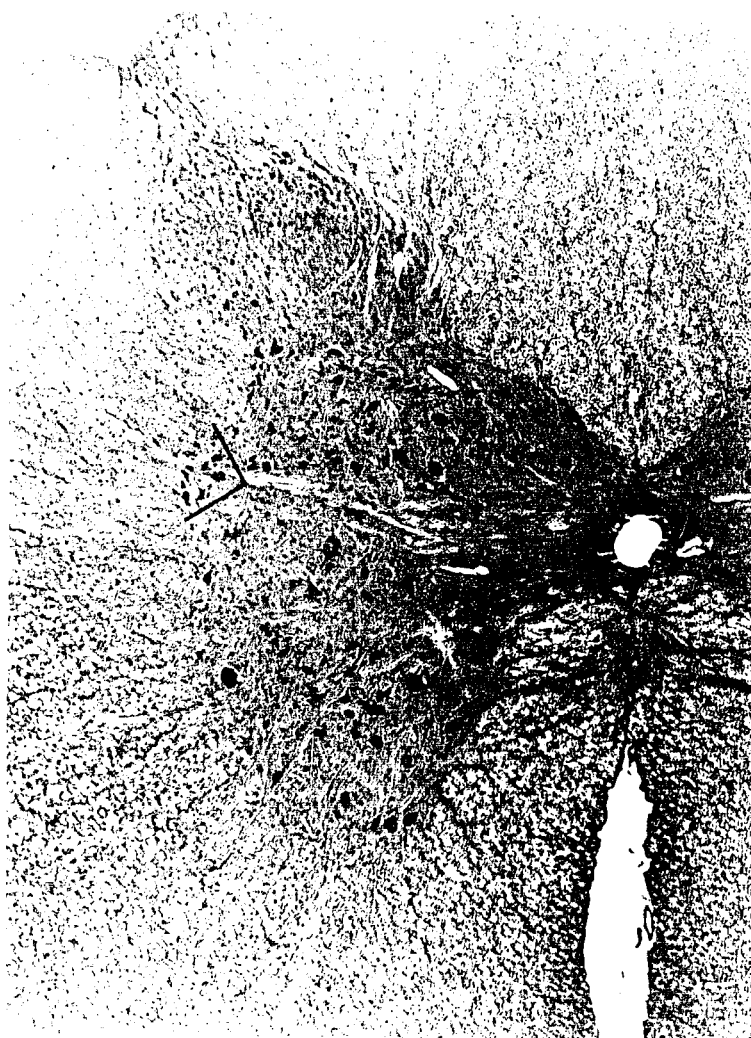
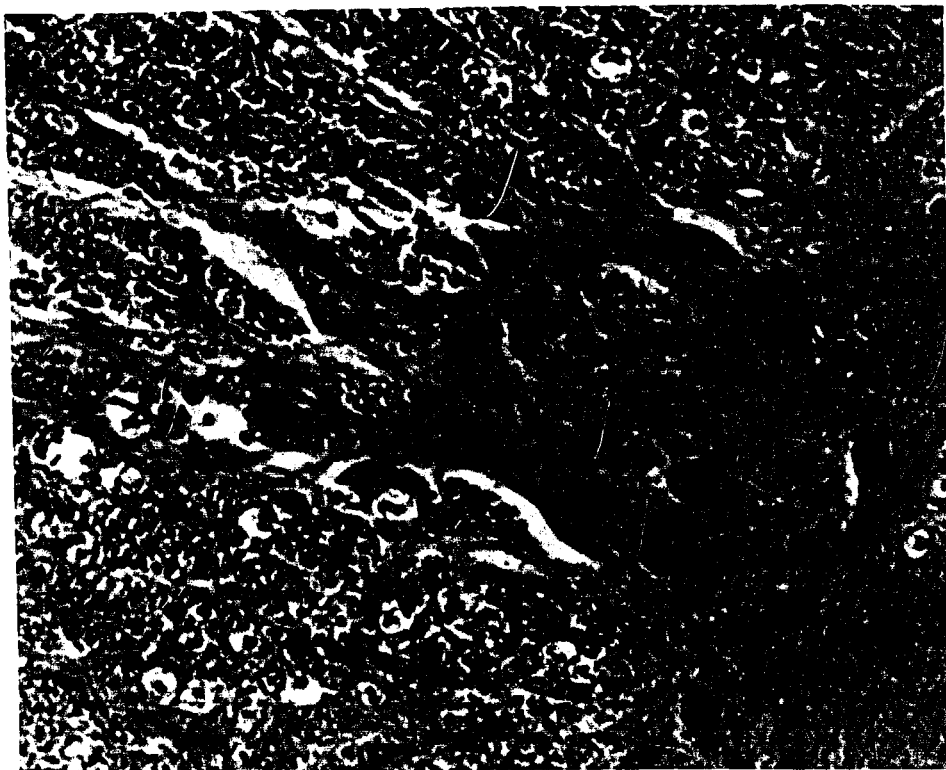


FIGURE 15

Transverse section of a portion of the spinal cord at the level of T₁ illustrating the distribution of neurons in finger-like projections of grey matter. In this section seven cells were counted. Calibration 50 μ m.



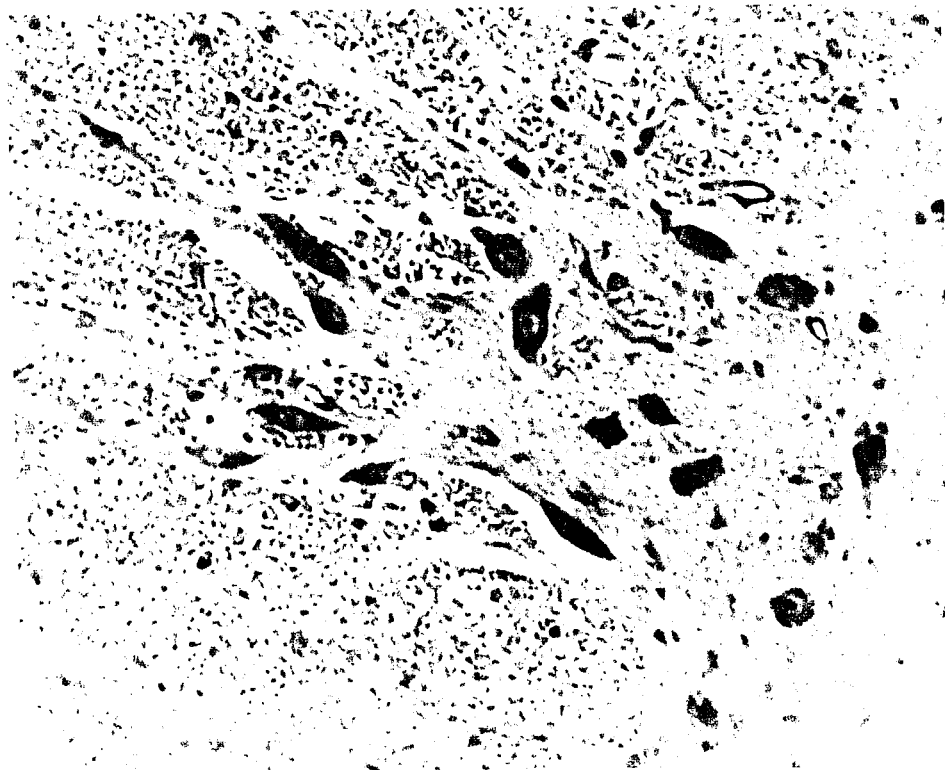
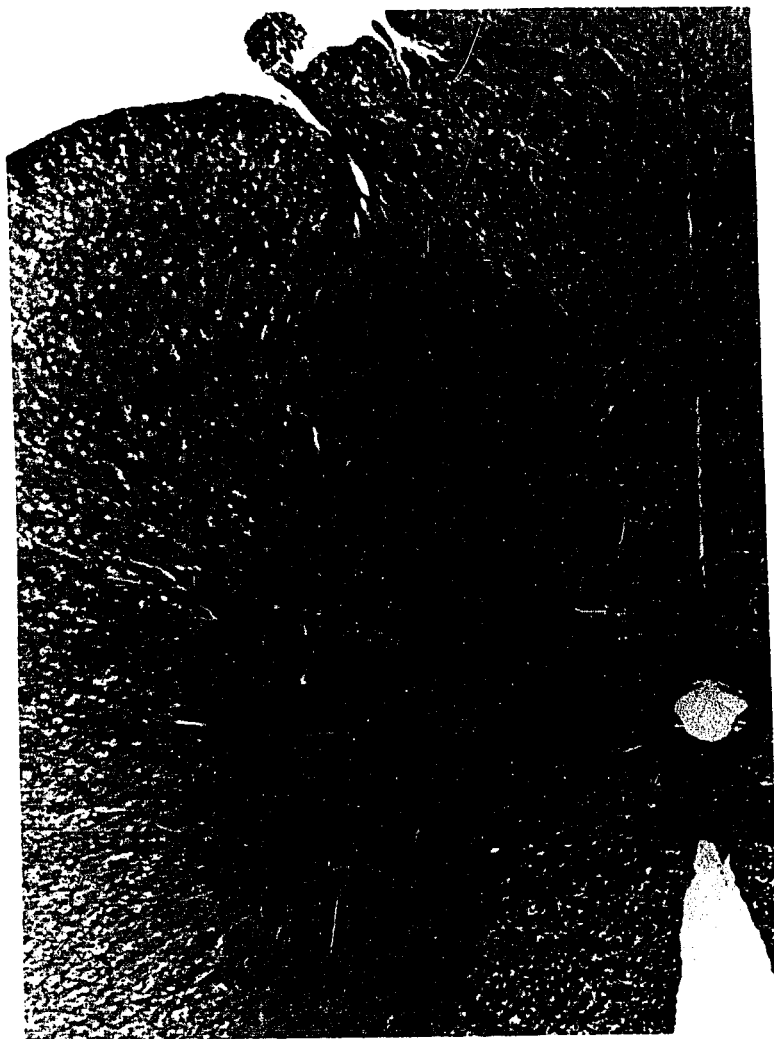


FIGURE 16

Transverse section of the spinal cord
at the level of T₁. The neurons
demarcated by the line were considered
to be in the ILN. Calibration 0.5 mm.



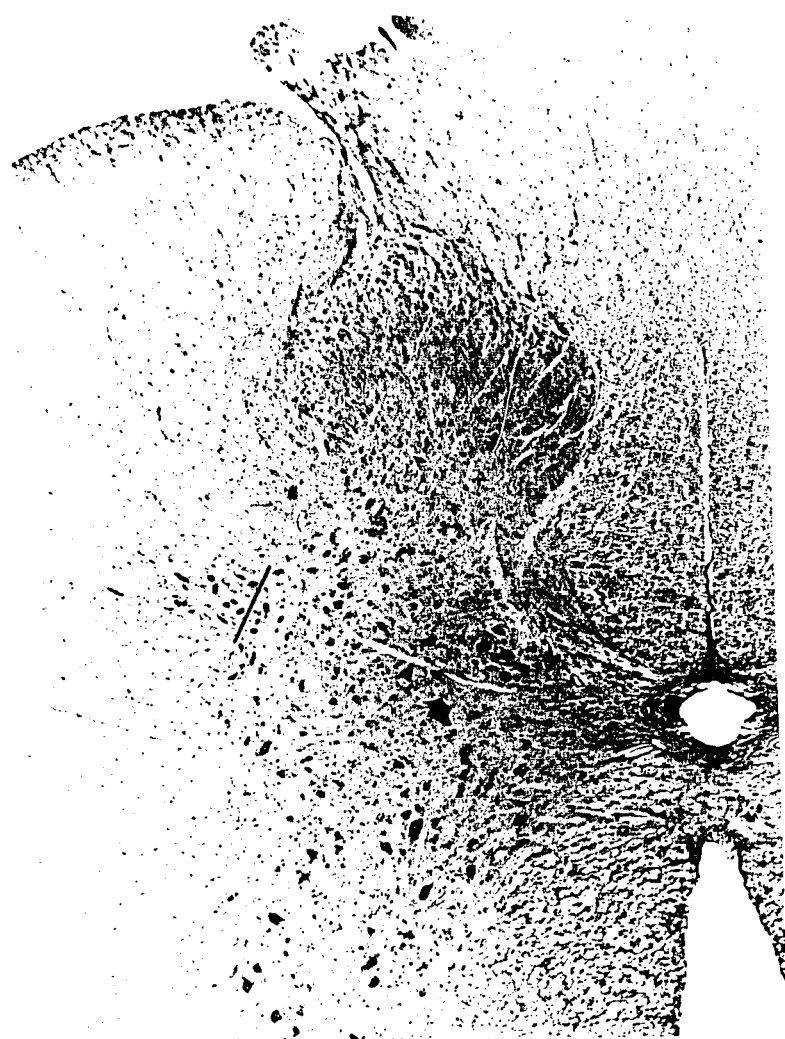


TABLE 5. ESTIMATED NUMBER OF SYMPATHETIC NEURONS IN THE THORACO-LUMBAR INTERMEDIOLATERAL NUCLEUS
IN EIGHT CATS.

Cat	Sex	Weights (kg)	Lengths of ILN (mm)	L. Side	R. Side	Total
9	F	3.0	192	16,350	16,440	32,790
12	F	2.2	177	18,250	19,320	37,570
13	F	3.0	216	18,880	19,470	38,350
15	F	2.8	209	16,220	17,240	33,460
Mean \pm SEM		2.75 \pm 0.19	198.5 \pm 8.8			35,543 \pm 1,411
11	M	2.6	206	26,760	26,580	53,340
14	M	3.0	230	21,310	21,990	43,300
16	M	2.5	189	20,170	22,000	42,170
17	M	2.4	180	21,490	22,860	44,350
Mean \pm SEM		2.62 \pm 0.13	201.5 \pm 11.0	19,929 \pm 1,207	20,738 \pm 1,166	45,765 \pm 2,556

(c) Distribution of Neurons

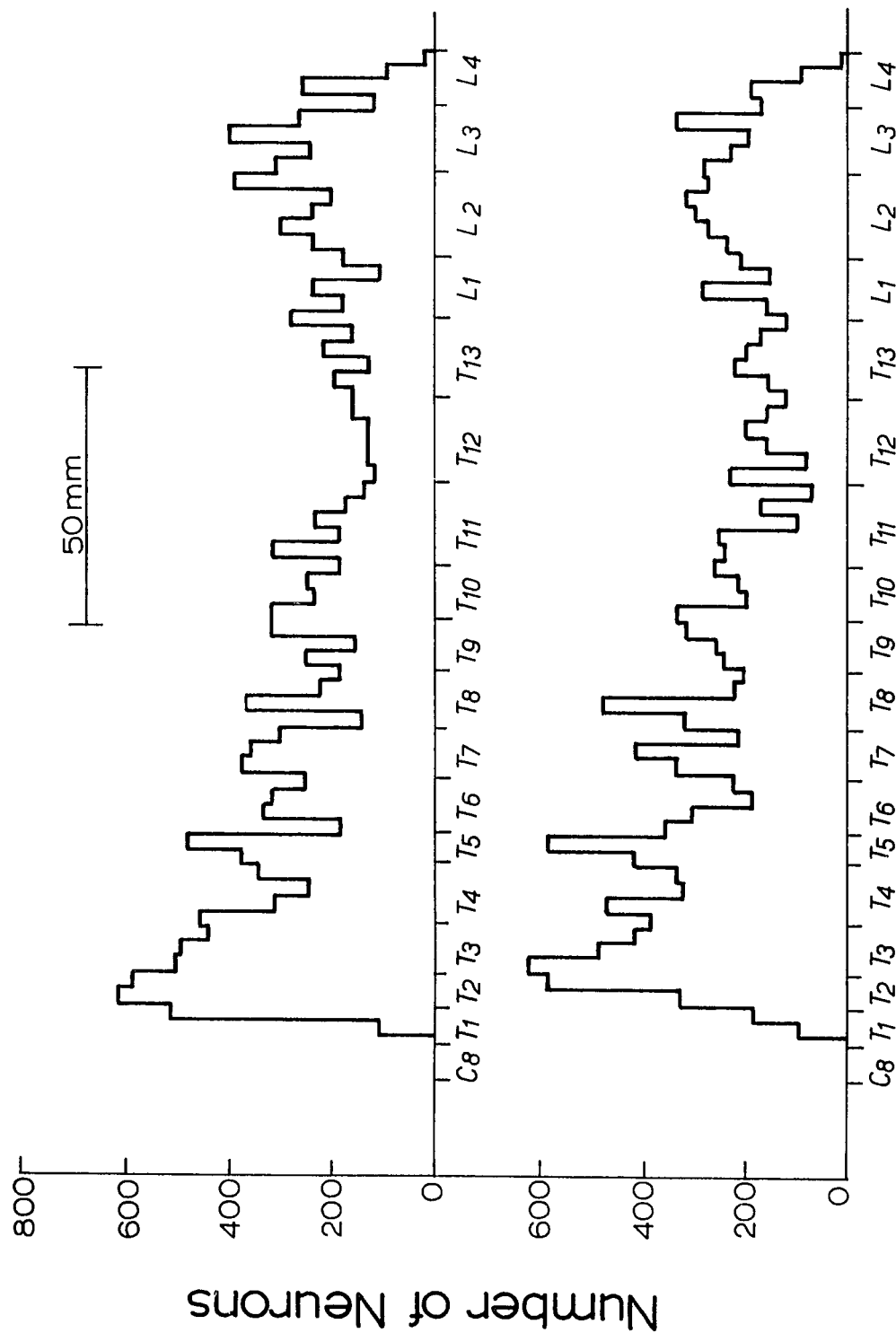
The number of ILN neurons varied from one section to another. Counts on each side of the cord ranged from 0 to 22 per section, with the majority (93%) of the sections containing less than nine neurons on each side. The segmental distribution was also variable with the greatest number of neurons at the levels of the rostral thoracic ($T_1 - T_2$) and of the middle lumbar ($L_3 - L_4$) segments. This distribution was consistent in the eight specimens examined and was observed on both sides of the spinal cord. The characteristic distribution of neurons in the ILN on the right and left sides of one cat is illustrated in Figure 17.

(d) Neuron Size

Estimated areas of the 369 ILN neurons measured were found to be distributed in unimodal fashion. They ranged from 100 to 500 μm^2 with the mode at 290 μm^2 . These results are shown in the histogram of Figure 18. In addition, 18 neurons with estimated areas ranging from 510 to 1020 μm^2 were found. As they were rarely encountered (approximately 5% of the total population examined) they were not included in the histogram.

FIGURE 17

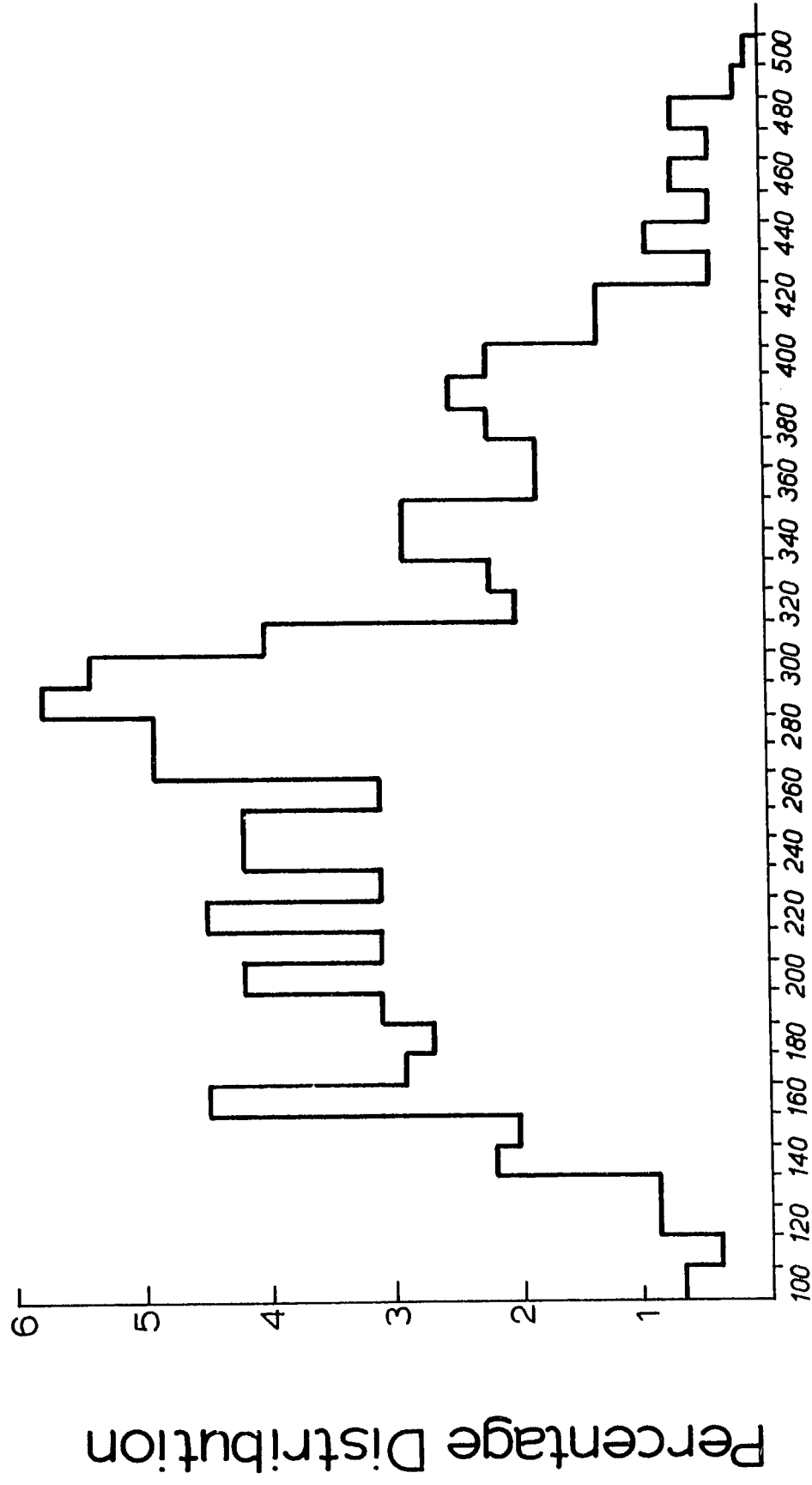
Number of ILN neurons in different segments of the spinal cord. Top panel, right side; bottom panel, left side. Note peaks of neuron counts at T₂ and at L₃ - L₄. Cat 9.



Spinal Cord Segment

FIGURE 18

Percentage distribution of estimated
cross-sectional areas of ILN neurons
measured in one specimen.



Quantitative Estimate of Area (μm^2)

B. Localization of Cardioacceleratory Sites in the ILN

(a) General

Of the 32 cats used in this series of experiments, electrical stimulation of the ILN was attempted in 28. The remaining four did not yield results because their condition deteriorated due to air emboli in the venous sinuses during laminectomy or because their general condition deteriorated due to bleeding or to trauma to the spinal cord.

Of the 28 cats in which electrical stimulation was attempted, reliable results were obtained from 20. In three of the remaining eight cats stimulation of the spinal cord failed to elicit a response; in five, responses to electrical stimulation were not of large enough magnitude to be used in this study. This was probably due to an overdose of nor-adrenaline. The effects of high rates of infusion of noradrenaline will be discussed in a later section.

In each of the 20 cats which yielded reliable results several penetrations aimed at the ILN were usually necessary to find a location from which electrical stimulation elicited cardiovascular responses. When a responsive site was found stimulation was repeated several times before the location of the electrode tip was marked.

As electrical stimulation of sympathetic nerves on the right side has been shown to elicit primarily an increase

in heart rate, while stimulation on the left elicits primarily an increase in contractile force (Randall & Rohse, 1956; Linden & Norman, 1969) preliminary experiments were done to obtain and compare responses to stimulation of the ILN on both sides of the mid line. The magnitudes of the cardioacceleration and arterial hypertension were found to be significantly greater during stimulation of the right side than during stimulation of the left side. It is particularly noteworthy that at the four sites stimulated on the left side no response was obtained at two sites, a minimal response at one and at the fourth site a response was obtained which was approximately one half of the characteristic response obtained from the right side. The data obtained are presented in Table 6. As the object of these experiments was to determine the longitudinal distribution of cardioacceleratory neurons within the ILN the following data include the results obtained during stimulation of sites on the right side only.

(b) Responses to Stimulation of the ILN

Selective electrical stimulation of 84 sites in the ILN in segments T₁ to mid-T₈ elicited cardioacceleration and arterial hypertension. Shifts in the position of the stimulating electrode of 0.5 mm in any plane resulted in disappearance of these responses. The increase in heart rate began with a mean latency of 2.3 ± 0.15 (SE) sec, reached a maximum value at 22.1 ± 0.81 sec and returned to pre-stimulus levels 125 ± 7.2

TABLE 6. INCREASES IN HEART RATE (HR) AND MEAN ARTERIAL PRESSURE (MAP) ELICITED BY ELECTRICAL STIMULATION OF THE INTER-MEDIOLATERAL NUCLEUS ON THE LEFT AND RIGHT SIDES OF THE SPINAL CORD.

Cat No.	Left Side			Right Side		
	Segment	HR, beats/min	MAP, mm Hg	Segment	HR, beats/min	MAP, mm Hg
206	T ₃	16	25	T ₄	39	48
206	T ₃	0	28	T ₄	30	47
207	T ₂	4	29	T ₂	31	34
207	T ₃	0	37	T ₂	33	39
Mean \pm SE		5.0 \pm 3.8	29.8 \pm 2.6		33.3 \pm 2.0*	42.0 \pm 3.3*

* Values on the right are significantly greater (t test; $P < 0.05$) than those on the left.

sec after termination of the stimulus. The increase in arterial pressure began with a mean latency of 1.6 ± 0.11 sec, reached a maximum value at 19.1 ± 0.69 sec and returned to pre-stimulus levels 73 ± 6.0 sec after termination of the stimulus. A characteristic response is shown in Figure 19.

The magnitudes of the responses were greatest during stimulation of sites in segments T_2 to T_6 . The responses could be elicited as far rostrally as the junction of segments C_8 and T_1 . The cardioacceleratory response became smaller in segment T_7 and could be elicited only as far caudally as the rostral half of segment T_8 . The arterial pressor response also became smaller in segment T_7 but could still be elicited from the most caudal points stimulated in segment T_9 . The mean magnitudes of the cardioacceleratory and arterial pressor responses obtained at the different segmental levels as well as the number of sites stimulated are shown in Figure 20.

(c) Effects of Administration of Gallamine Triethiodide

To exclude the possibility that the cardiovascular changes elicited by electrical stimulation of the ILN were reflex adjustments secondary to motor activity caused by stimulus spread to motor pathways, at five sites from which cardiovascular responses were elicited the animals were paralyzed by the administration of gallamine triethiodide (Flaxedil, Poulenc, Montreal; 5 mg/kg intravenously) and the magnitudes of the res-

FIGURE 19

Typical cardioacceleratory and arterial hypertensive response elicited by stimulation of the thoracolumbar intermediolateral nucleus on the right side of the spinal cord. A and B are continuous records. In each panel the top tracing is the tachograph record; the middle tracing is arterial pressure; the stimulus was applied between ON and OFF.

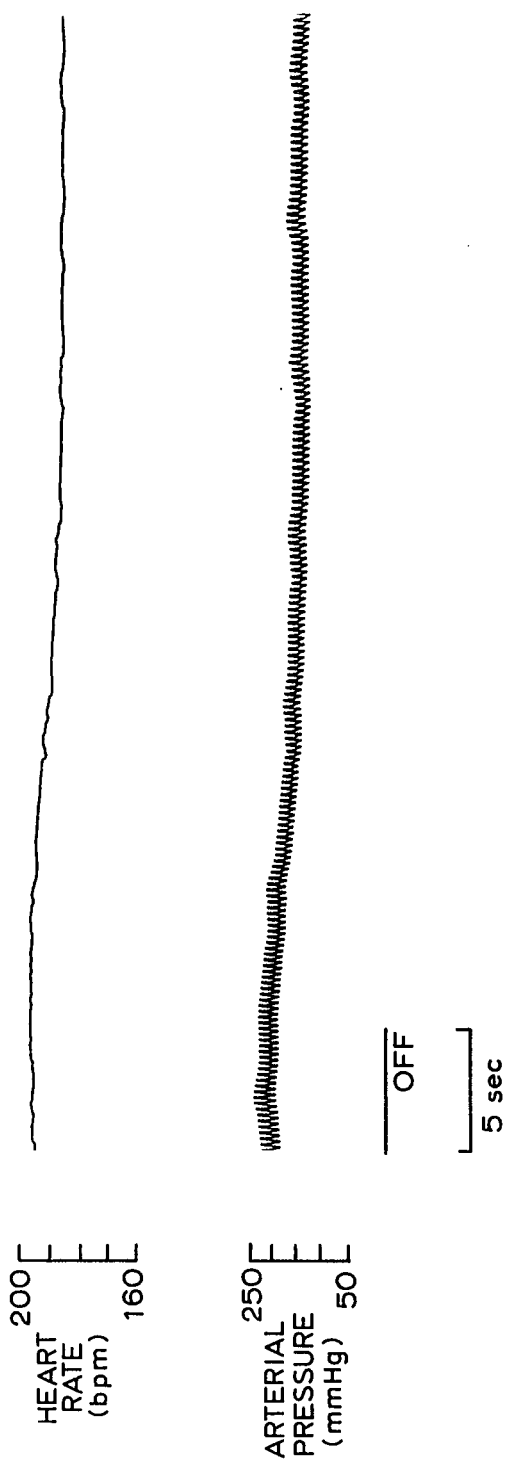
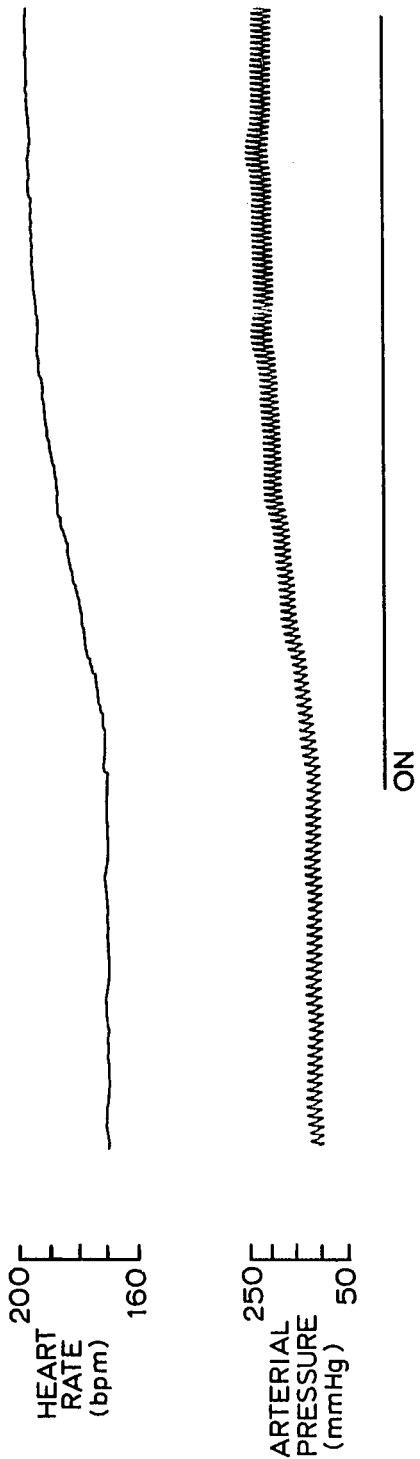
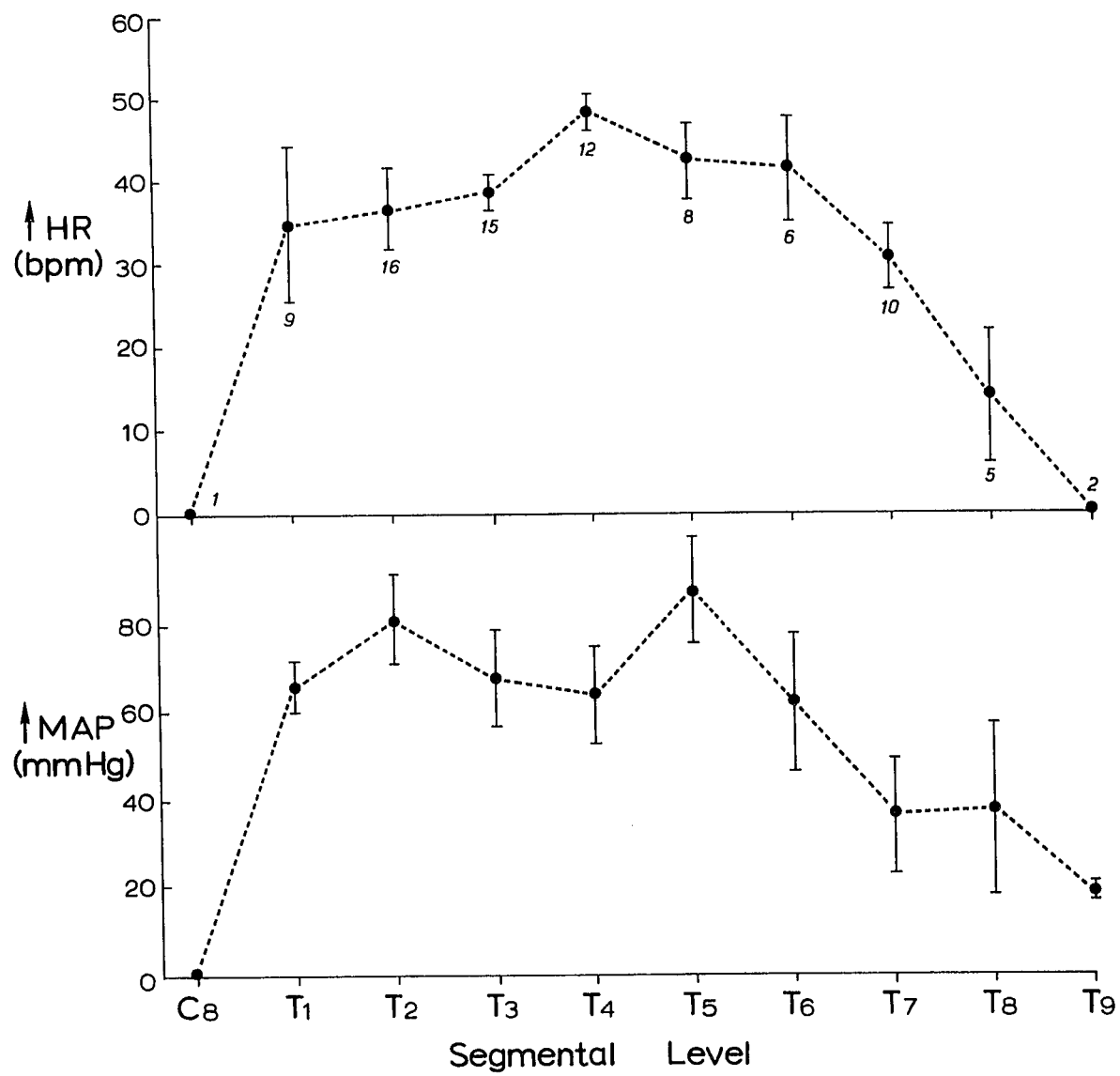


FIGURE 20

Increases in heart rate (HR) and mean arterial pressure (MAP) elicited by stimulation of the intermediolateral nucleus at different segmental levels. At each level, mean increase \pm SE and number of sites stimulated.



ponses before and after administration of the drug were compared. The animals were artificially ventilated and end-expired CO_2 concentration was monitored on a Beckman LBI infrared CO_2 meter to maintain the end-expired CO_2 concentration between 3 and 5%. Administration of gallamine triethiodide had no significant effect ($p < 0.05$) on either the cardioacceleratory or the arterial pressor responses. These experiments are summarized in Table 7.

(d) Effect of Stimulus Frequency on the Magnitude of the Response

At four active sites in the ILN experiments were done to determine the relation between stimulus frequency and the magnitude of the cardiovascular responses. In Figure 21, illustrating the relation between heart rate and frequency of stimulation to the ILN, it can be seen that the greatest change in the magnitude of the response for a change in stimulus frequency occurred in the range up to approximately 5 Hz, and that maximum cardioacceleration was elicited with a stimulus frequency of 25 Hz. The relation of the pressor response to the stimulus frequency, also illustrated in Figure 21, is similar to that of the heart rate except that the greatest change in the magnitude of the response for a change in stimulus frequency occurred in the range up to approximately 20 Hz, and the maximum response occurred at a frequency of 20 Hz.

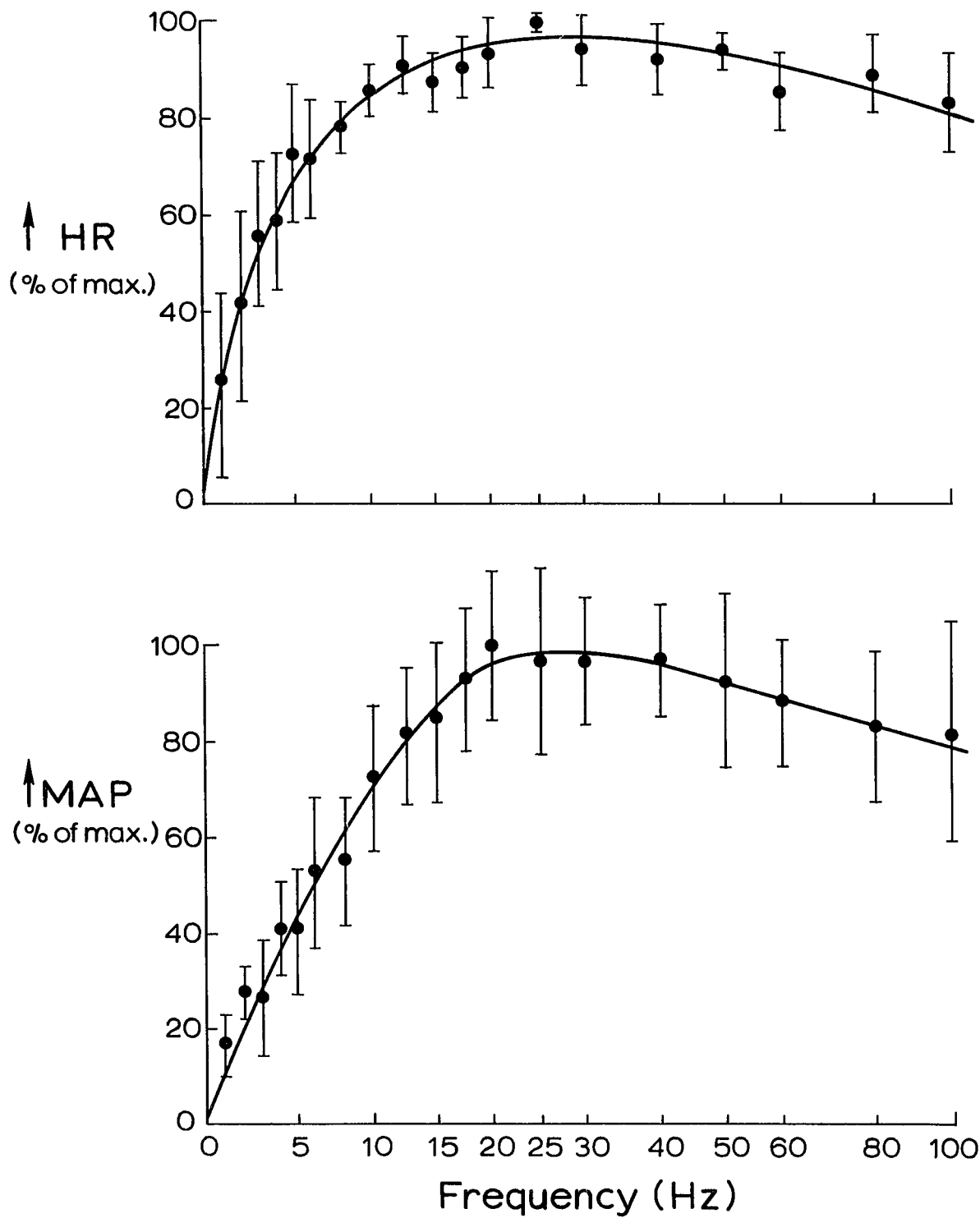
TABLE 7. INCREASES IN HEART RATE AND MEAN ARTERIAL PRESSURE ELICITED BY ELECTRICAL STIMULATION OF THE INTERMEDIOLATERAL NUCLEUS BEFORE AND AFTER THE ADMINISTRATION OF GALLAMINE TRIETHIODIDE (5 mg/kg intravenously).

Cat No.	Segment	Heart Rate, Beats/min		Mean Arterial Pressure, mm Hg	
		Before	After	Before	After
196	T ₂	34	38	112	130
203	T ₃	55	51	21	18
205	T ₃	42	57	75	72
205	T ₅	25	24	52	40
205	T ₅	50	48	85	99
Mean \pm SE		41.2 \pm 5.4	43.6 \pm 5.8*	69.0 \pm 15.4	71.7 \pm 20.0*

* Values are not significantly different ($P < 0.05$) from those before gallamine.

FIGURE 21

Increases in heart rate (HR) and mean arterial pressure (MAP) elicited by stimulation of the intermediolateral nucleus at different frequencies of stimulation. At each frequency, mean \pm SE of responses from four sites.



C. Sites of Origin of Descending Cardioacceleratory Pathways
from the Medulla to the ILN

Of the 29 cats used in this series of experiments 22 gave reliable results. In two of the remaining seven cats stimulation of the ILN failed to elicit a response in the medulla and five did not yield results because their general condition deteriorated due to bleeding or swelling of the brain stem or to other causes.

(a) Characteristics of the evoked responses from the
medulla

In the 22 cats from which reliable results were obtained 216 penetrations were made within the region of the medulla explored. (see Figure 7). Evoked activity was recorded at 266 sites. The responses were of the monophasic, biphasic or triphasic types. Records of superimposed sweeps of each type of response are shown in Figure 22. As the electrode was advanced in the vicinity of an active site the amplitude of the evoked responses changed and the characteristics of the responses were measured at the point of maximum amplitude. The values obtained for onset and peak latency, duration and peak-to-peak amplitude are presented in Table 8. As each response approached its maximum amplitude the rising slope became steeper and the peak latency decreased.

FIGURE 22

Typical evoked responses recorded from sites in the medulla during selective electrical stimulation of the ILN.

Each record is composed of three superimposed sweeps. Time calibration in each record is 1 msec.

100 μ V
-
+

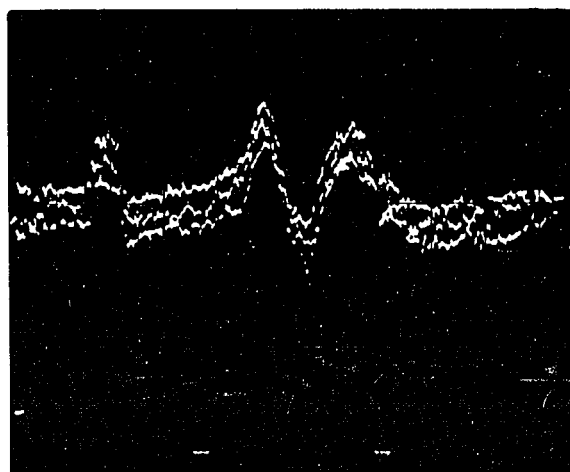
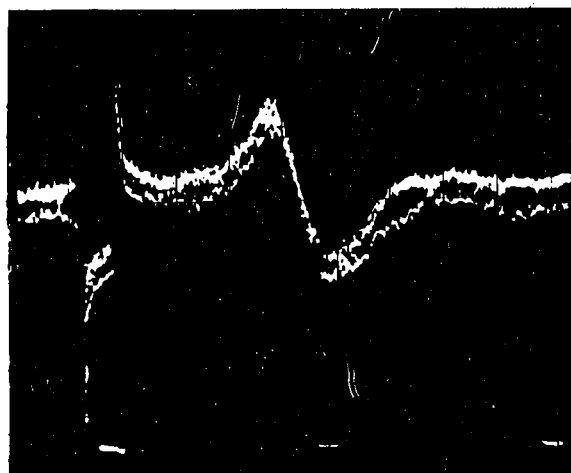
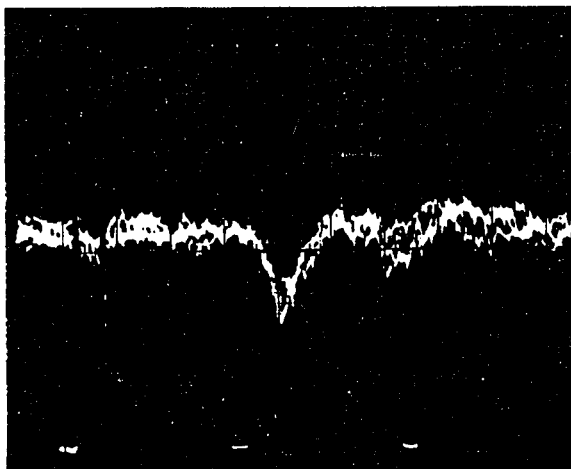


TABLE 8. CHARACTERISTICS OF THE EVOKED RESPONSES RECORDED FROM
THE MEDULLA DURING ELECTRICAL STIMULATION OF THE ILN.

Form	Monophasic, biphasic, or triphasic
Onset Latency (msec)	
Mean \pm S.E.M.	1.5 \pm 0.04
Range	0.75 - 4.25
Peak Latency (msec)	
Mean \pm S.E.M.	1.8 \pm 0.04
Range	1.0 - 5.25
Duration (msec)	
Mean \pm S.E.M.	1.0 \pm 0.03
Range	0.25 - 3.5
Peak to Peak Amplitude (μ V)	
Mean \pm S.E.M.	129 \pm 4.2
Range	25 - 375

(b) Localization of the sites from which evoked responses were recorded

The distribution of sites from which evoked responses were recorded is illustrated in Figure 23 in schematic diagrams of transverse sections of the medulla of the cat. Responses were localized to the following structures: raphé NN. (55 sites), N. medullae oblongatae centralis subnucleus ventralis (41), N. lateralis reticularis (35), N. paramedium reticularis (23), N. gigantocellularis (21), inferior olivary N. (19), N. medullae oblongatae centralis subnucleus dorsalis (18), N. parvocellularis (15), N. pontis centralis caudalis (7), N. interpositus hypoglossi (7), and N. retroambiguus (4). Twenty-one sites were in non-nuclear regions. The medullary nuclei from which evoked responses were recorded are listed in Table 9 along with the number of sites observed in each structure.

The temporal characteristics of the responses were determined for each of the structures from which fifteen or more evoked potentials were recorded. The peak latency, rather than the onset latency was used for making comparisons as it could be more precisely measured, and it was found to vary in different structures. The mean peak latency and the mean duration for the responses recorded from each of these structures is presented in Table 10. No attempt was made to determine whether the amplitudes of the responses varied from structure to structure because the

FIGURE 23

Cross-sectional drawings of the medulla of the cat at 1 mm intervals illustrating the sites in the medulla at which evoked responses were recorded during electrical stimulation of a cardioacceleratory site in the ILN. Calibration = 5 mm. The abbreviations used to label the structures, in the medulla were from Taber (1961), and are listed below.

A	-	N. ambiguus
C	-	N. cuneatus
Cd	-	N. medullae oblongatae centralis subnucleus dorsalis
Cv	-	N. medullae oblongatae centralis subnucleus ventralis
G	-	N. gracilis
Gc	-	N. gigantocellularis
Ic	-	N. intercalatus
Ih	-	N. interfascicularis hypoglossi
L	-	N. lateralis reticularis
Oi	-	inferior olivary N.
Os	-	superior olivary N.
Pc	-	N. parvocellularis
Pm	-	N. paramedium reticularis
Poc	-	N. pontis centralis caudalis
Prp	-	N. praepositus hypoglossi
R	-	N. retroambiguus
Rf	-	N. retrofacialis
Rob	-	N. raphé obscurus
Rpa	-	N. raphé pallidus
Rm	-	N. raphé magnus
S	-	N. of tractus solitarius
V	-	N. tractus spinalis trigemini
VII	-	facial N.
VIII	-	N. vestibularis
X	-	dorsal N. of the vagus
XII	-	hypoglossal N.

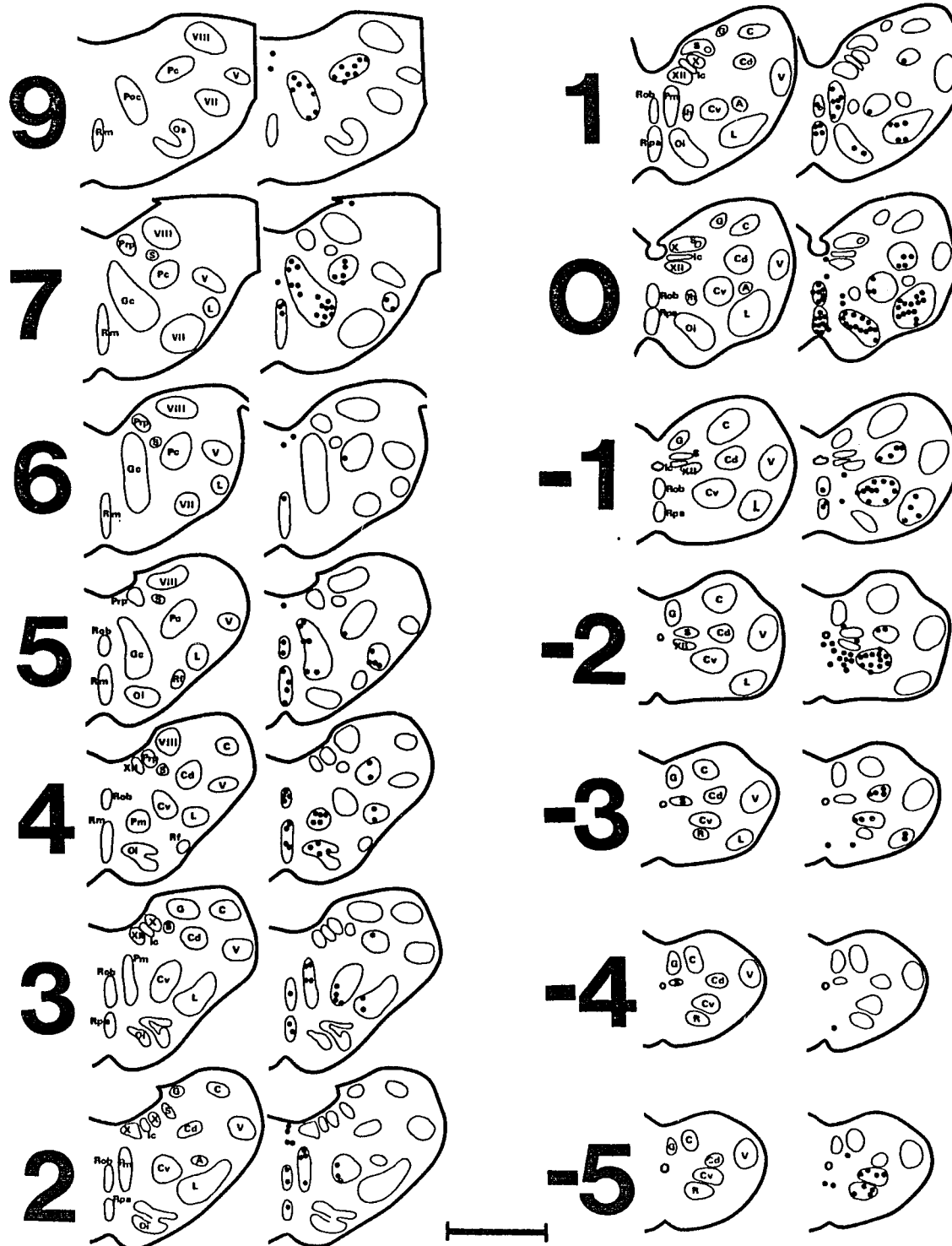


TABLE 9. LOCATIONS OF SITES IN THE MEDULLA FROM WHICH EVOKED ACTIVITY
WAS RECORDED DURING ELECTRICAL STIMULATION OF THE ILN.

Location	No. of Sites	% of Total No.
raphé NN.	55	20.68
N. medullae oblongatae centralis		
subnucleus ventralis	41	15.41
N. lateralis reticularis	35	13.16
N. paramedium reticularis	23	8.65
N. gigantocellularis	21	7.89
inferior olivary N.	19	7.14
N. medullae oblongatae centralis		
subnucleus dorsalis	18	6.77
N. parvocellularis	15	5.64
N. pontis centralis caudalis	7	2.63
N. interfascicularis hypoglossi	7	2.63
N. retroambiguus	4	1.50
non-nuclear regions	21	7.89
Total	266	

TABLE 10. MEAN (\pm SE) PEAK LATENCIES AND MEAN DURATIONS OF THE
EVOKED RESPONSES RECORDED FROM EACH STRUCTURE IN THE
MEDULLA.

Nucleus	Mean Peak Latency (msec)	Mean Duration (msec)	No. of Sites
N. medullae oblongatae centralis subnucleus dorsalis	2.0 ± 0.12	1.1 ± 0.12	18
subnucleus ventralis	1.7 ± 0.11	1.1 ± 0.01	41
N. gigantocellularis	1.7 ± 0.13	1.0 ± 0.08	21
N. parvocellularis	1.5 ± 0.23	1.2 ± 0.13	15
N. lateralis reticularis	1.4 ± 0.10	0.9 ± 0.06	35
raphé NN.	1.4 ± 0.08	0.9 ± 0.07	55
N. paramedium reticularis	1.3 ± 0.10	0.8 ± 0.11	23
inferior olivary N.	1.0 ± 0.07	0.6 ± 0.05	19

evoked responses were not necessarily recorded from the centre of the sink or source.

(c) Effects of repetitive stimulation on the evoked responses from the medulla

At each site in the medulla from which an evoked potential was recorded the effect on the response of high frequencies of stimulation was investigated. All responses included in the results were observed at frequencies of stimulation greater than 200 Hz. The configuration and the amplitude of the responses remained constant at all frequencies.

(d) Effects of asphyxia and barbiturates on the evoked responses from the medulla

At seven sites in four animals the effect of occlusion of the tracheal cannula (asphyxia) on the evoked responses recorded from the medulla was determined. At each site the latency of the initial deflection remained unchanged after 90 - 150 sec of tracheal occlusion. The results obtained at one site are illustrated in Figure 24. At two sites in two animals initially anaesthetized with 60 mg/kg of alpha chloralose the effect of an overdose of barbiturate anaesthetic on the evoked responses was determined. Each animal was given four individual doses of 5 mg/kg of sodium pentobarbital intravenously, each dose following

FIGURE 24

Records showing the effects of hypoxia on an evoked response recorded from a site in the medulla during selective electrical stimulation of the ILN. Each record consists of three sweeps.

DURATION
OF HYPOXIA
(sec)

EVOKED RESPONSE
FROM MEDULLA

CONTROL

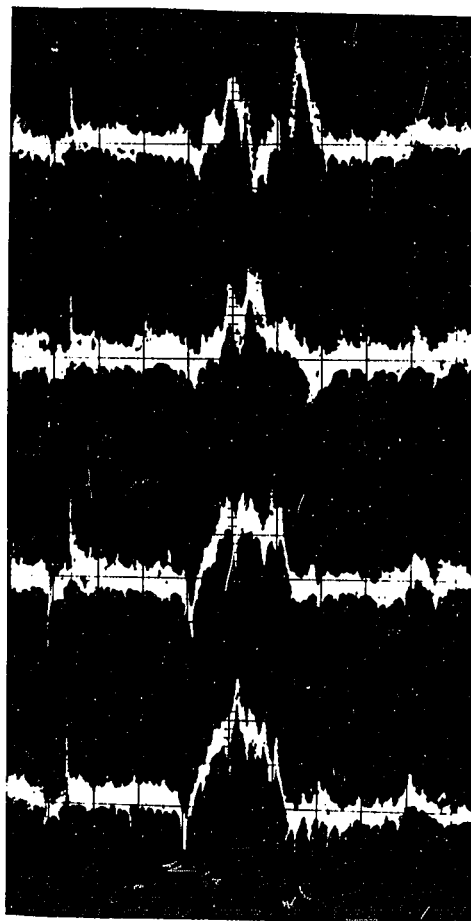
45

90

150

100 μ V

1msec



the previous one by an interval of five min. In both cases the animals stopped breathing spontaneously between the second and third doses and had to be artificially ventilated. The responses were not changed by the administration of the barbiturate. The results from one experiment are illustrated in Figure 25.

(e) Determination of the size of the stimulus field in the spinal cord

The size of the stimulus field in the spinal cord was tested at three sites in two animals. In each case, once a site had been obtained from which an evoked response was recorded, the effects on the response of slight dorsoventral or mediolateral movement of the stimulating electrode were determined. Movement of the spinal electrode of 0.5 mm in either direction led to an abolition of the response from the medulla. The results from one experiment are illustrated in Figure 26.

(f) Effects of stimulus strength on the evoked responses from the medulla

At each site from which evoked activity was recorded the effect on the response of changing the stimulus voltage between 3 and 12 V was determined. All responses maintained constant onset and peak latencies and constant duration throughout the range of voltages used but the amplitudes of most responses varied directly

SODIUM
PENTOBARBITAL
(mg/kg)

EVOKED RESPONSE
FROM MEDULLA


CONTROL

5

10

15

20

100 μ V 
msec

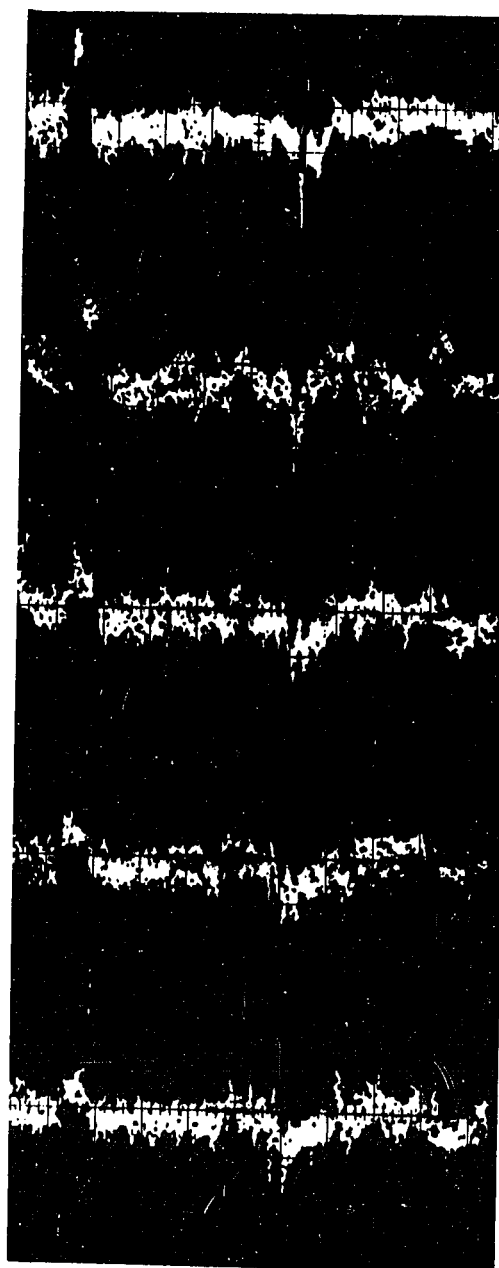


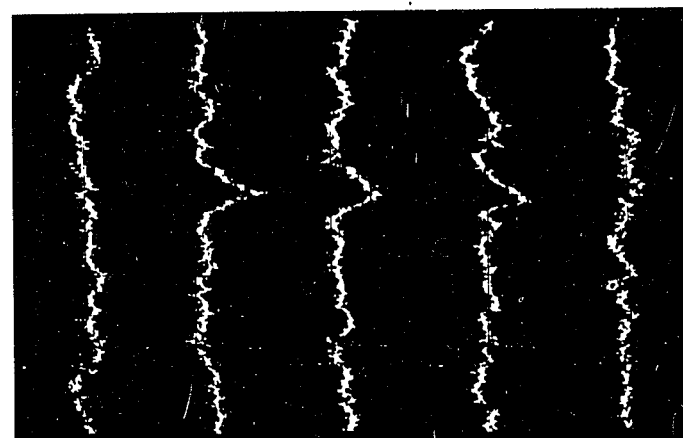
FIGURE 26

Records showing the effects of dorsoventral movement of the spinal electrode on an evoked response recorded from the medulla during selective electrical stimulation of the ILN. Each record consists of a single sweep.

DEPTH OF
SPINAL ELECTRODE

EVOKED RESPONSE
FROM MEDULLA

(mm)



- 1.5

- 1.75

- 2.0

- 2.25

- 2.5

100 μ V

1msec

as the voltage.

- (g) Effects on heart rate and arterial pressure of electrical stimulation of medullary sites from which evoked responses were recorded

To determine whether the medullary structures implicated in this study projected to cardioacceleratory neurons or to ILN neurons mediating other autonomic functions, attempts were made to determine the effects on heart rate and arterial pressure of electrical stimulation of 81 of the sites from which evoked responses were recorded. The parameters of stimulation were similar to those used for stimulation of the spinal cord. Typical cardiovascular responses are shown in Figure 27. The locations of the sites stimulated are illustrated along with the cardiovascular changes in Figure 28.

Cardioacceleration was elicited from 17 sites localized primarily to the N. lateralis reticularis and to the N. parvocellularis. Cardiac slowing was elicited from 29 sites localized primarily to the raphe NN., the N. paramedium reticularis and the N. medullae oblongatae centralis subnucleus ventralis. Stimulation of the remaining 35 sites did not produce changes in heart rate. The changes in heart rate occurring during electrical stimulation of medullary sites are summarized in Table 11.

An increase in arterial pressure was elicited by stimulation of 11 sites primarily in the N. lateralis reticularis and the N. parvocellularis. A decrease in arterial pressure was elicited by

FIGURE 27

Typical changes in heart rate and in arterial pressure during electrical stimulation of sites in the medulla from which evoked responses were recorded. A. Response observed during electrical stimulation of a site in the N. lateralis reticularis. B. Response observed during electrical stimulation of a site in the raphé NN. In each panel the top tracing is the tachograph record; the middle tracing is arterial pressure; each stimulus was applied between ON and OFF.

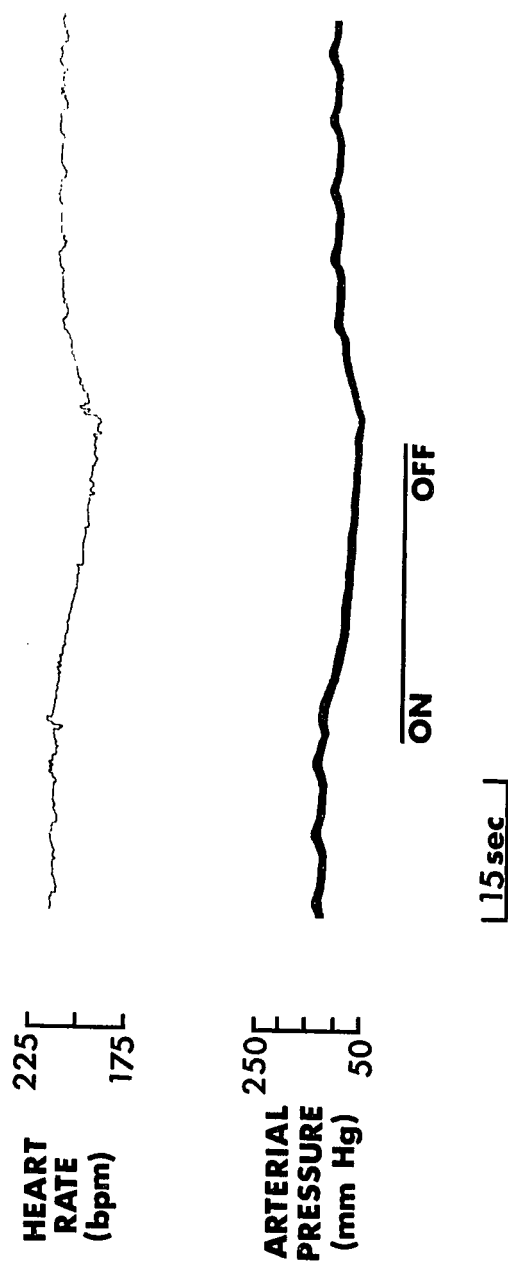
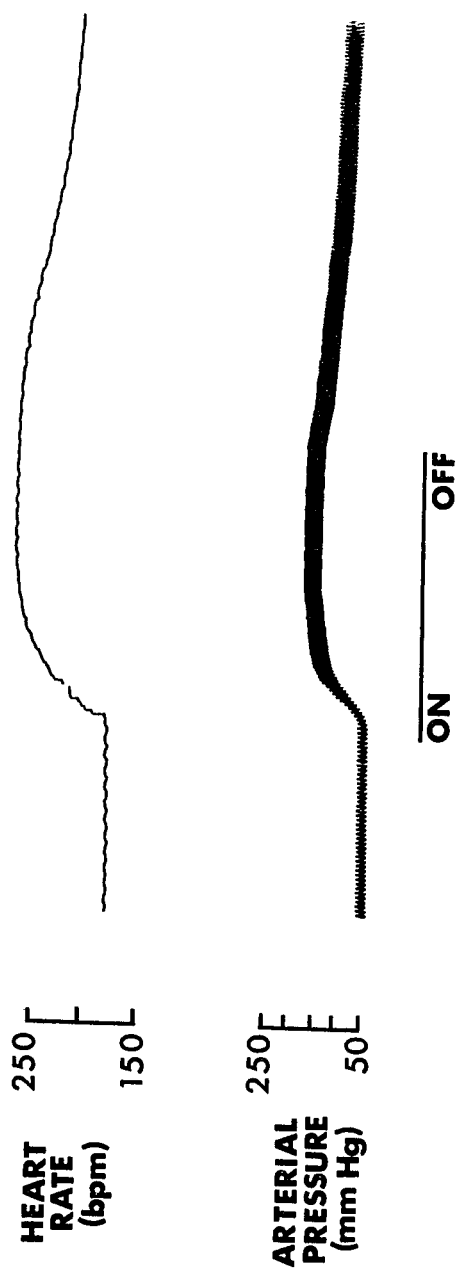


FIGURE 28

Cross-sectional drawings of the medulla of the cat at 1 mm intervals illustrating the changes in heart rate and arterial pressure of electrical stimulation of sites at which evoked responses were recorded. See Figure 23 for identification of structures. Calibration = 5 mm.

11 ■ = cardioacceleration and hypertension

6 ▲ = cardioacceleration

29 □ = cardiac slowing and hypotension

10 △ = hypotension

25 ● = no response

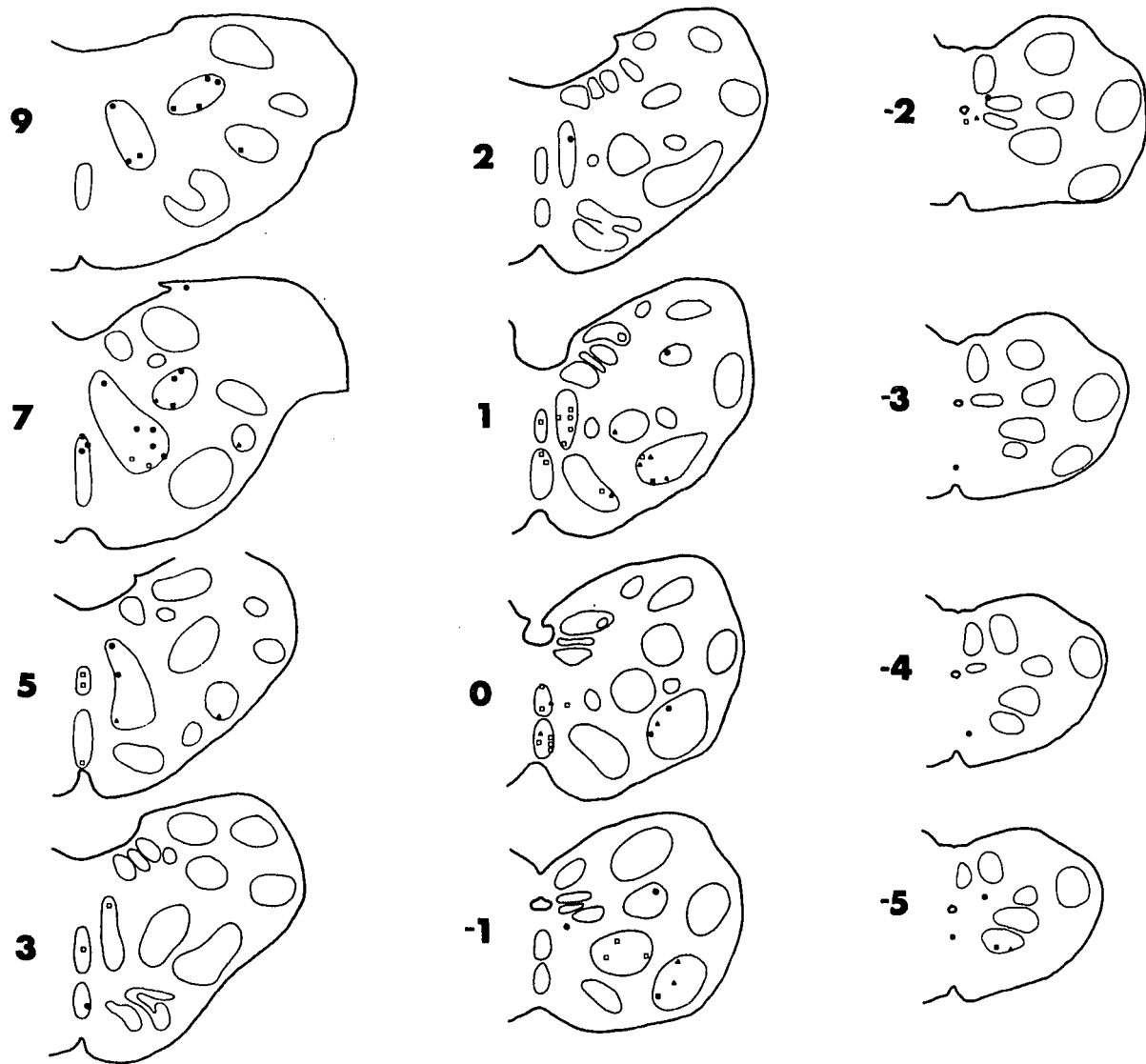


TABLE 11. EFFECTS ON HEART RATE (HR) OF ELECTRICAL STIMULATION OF 81
OF THE SITES IN THE MEDULLA FROM WHICH EVOKED RESPONSES
WERE RECORDED DURING ELECTRICAL STIMULATION OF THE ILN.

	Location	No. of Sites Eliciting HR Response	No. of Sites Stimulated
↑ HR	N. lateralis reticularis	9	14
	N. parvocellularis	4	8
	N. pontis centralis caudalis	1	3
	inferior olivary N.	1	2
	N. retroambiguus	1	2
	non-nuclear regions	1	7
↓ HR	raphé NN.	14	21
	N. paramedium reticularis	8	8
	N. medullae oblongatae centralis		
	subnucleus ventralis	3	4
	N. gigantocellularis	2	10
	N. lateralis reticularis	1	14
	inferior olivary N.	1	2
- HR	N. gigantocellularis	8	10
	raphé NN.	7	21
	N. lateralis reticularis	4	14
	N. parvocellularis	4	8
	N. pontis centralis caudalis	2	3
	N. medullae oblongatae centralis		
	subnucleus dorsalis	2	2
	subnucleus ventralis	1	4
	N. retroambiguus	1	2
	non-nuclear regions	6	7

stimulation of 39 sites primarily in the raphé NN., the N. paramedial reticularis and the N. medullae oblongatae centralis subnucleus ventralis. Stimulation of the remaining 31 sites produced no change in arterial pressure. The changes in arterial pressure occurring during electrical stimulation of medullary sites are summarized in Table 12.

(h) Responses recorded from the contralateral medulla

In three cats 27 penetrations were made in the contralateral medulla in search of evoked responses during electrical stimulation of the ILN on the right side. Evoked activity was recorded at four sites, two in the N. medullae oblongatae centralis and two in the raphé NN. The responses were similar temporally and in configuration to those recorded from the medulla on the right side. Each response persisted at stimulus frequencies of greater than 200 Hz. Electrical stimulation of the sites in the raphé NN. elicited cardiac slowing and arterial hypotension, while stimulation of the sites in the N. medullae oblongatae centralis produced no change in heart rate or arterial pressure at one site and a decrease in arterial pressure alone at the other site.

TABLE 12. EFFECTS ON ARTERIAL PRESSURE (AP) OF ELECTRICAL STIMULATION
OF 81 OF THE SITES IN THE MEDULLA FROM WHICH EVOKED RESPONSES
WERE RECORDED DURING ELECTRICAL STIMULATION OF THE ILN.

	Location	No. of Sites Eliciting the Response	No. of Sites Stimulated
↑ AP	N. lateralis reticularis	5	14
	N. parvocellularis	3	8
	N. pontis centralis caudalis	1	3
	N. retroambiguus	1	2
	non-nuclear regions	1	7
↓ AP	raphé NN.	17	21
	N. paramedium reticularis	8	8
	N. lateralis reticularis	5	14
	N. medullae oblongatae centralis subnucleus ventralis	4	4
	N. gigantocellularis	3	10
	inferior olivary N.	1	2
	N. retroambiguus	1	2
- AP	N. gigantocellularis	7	10
	N. parvocellularis	5	8
	N. lateralis reticularis	4	14
	raphé NN.	4	21
	N. pontis centralis caudalis	2	3
	N. medullae oblongatae centralis subnucleus dorsalis	2	2
	inferior olivary N.	1	2
	non-nuclear regions	6	7

DISCUSSION

The results of this investigation will be discussed in three sections corresponding to the three projects completed.

A. Topography and Numerical Distribution of ILN Neurons

Information has been presented on the topography of the ILN and estimates have been obtained of the numerical distribution and cross-sectional area of sympathetic neurons in the spinal cord of the cat.

The rostral pole of the ILN in the eight specimens studied was observed at the junction of segments C₈ and T₁, a finding in general agreement with the results of previous workers. Takahashi (1913) and Laruelle (1937) in the cat, and Jacobsohn (1908), Massazza (1923 a, b, 1924), Bok (1928), Gagel (1928, 1932), Greving (1928), Laruelle (1937) and Riley (1960) in man, located the rostral pole in the eighth cervical segment; Rexed (1954) in the cat and Navaratnam & Lewis (1970) in the rat located it in the first thoracic segment. The disagreement between these two groups of authors may be explained either on the basis of a species difference, a difference in the definition of the limits of C₈ and T₁, or on the difficulty of observing small numbers of neurons scattered throughout the finger-like projections of grey matter.

There is less agreement regarding the location of the caudal pole of the ILN. In the cat it has been found at the level of L₁

(Takahashi, 1913), L₂ (Laruelle, 1937) and L₄ (Rexed, 1954). In man it has been found at the level of either L₂ (Gagel, 1928, 1932; Laruelle, 1937) or L₃ (Jacobsohn, 1908, Greving, 1928; Riley, 1960), or to merge with the sacral parasympathetic lateral horn (Bok, 1928; Massazza, 1923 a, b, 1924). In the rat it has been found to be in L₂ - L₃ (Navaratnam & Lewis, 1970). With regard to the termination of the ILN in species other than the cat it appears pointless to make comparisons because of the possibility of variation between species. In the cat, however, the disagreement between the findings of the present study, indicating that the ILN terminates at L₄, and those of Takahashi, Laruelle and Rexed, indicating that the ILN terminates at L₁ - L₂, may be explained by the finding that the lateral horn disappears and the ILN becomes less distinct at these levels.

A novel finding in this investigation was that of the finger-like projections of grey matter extending into the white matter at the levels of C₈ - T₁ and L₁ - L₂. As the appearance of these projections was completely different from the usual rounded appearance of the lateral border of the lateral horn, the possibility was entertained that at these two locations the neurons observed were not part of the ILN. However, as these neurons were similar morphologically to the ILN neurons in the lateral horn, and as there was a gradual transition in the position of these cells from the lateral horn to the finger-like projections, they were included in the counts. The functional and anatomical significance of this unusual arrangement of neurons observed in all animals studied is not readily apparent.

The variability in the number of neurons from one section to another was a consistent finding throughout the length of the ILN. This uneven longitudinal distribution has been referred to by Rexed (1954) as "beading", which suggests that the ILN is a cylindrical column interrupted by beads or irregular expansions. The observations in this study indicate that the cross-sectional area of the lateral horn did not change markedly even when a large change in the number of neurons was noted. It is therefore suggested that the term "beading" should imply only that the number of ILN neurons varies from section to section without a change in cross-sectional area of the lateral horn.

The rostral thoracic and middle lumbar segments were observed to contain a greater number of neurons than other regions of the ILN. As these segments correspond to the cervical and lumbar enlargements, known to contain cell bodies of axons destined to the limbs (Weil & Lassek, 1929) it is suggested that they are the sites of origin of the sympathetic outflow to the limbs.

The information presented on the segmental distribution of neurons in the ILN is useful for experiments in which intracellular or extracellular single unit recording from the ILN is attempted because it gives an indication of the relative probability of encountering ILN neurons at the different segmental levels. It must be mentioned, however, that because of the "beading" in the column of ILN neurons this information will be of limited value in any one penetration.

The remarkable agreement between the counts on the two sides of the cord demonstrates a symmetrical spinal representation of the sympathetic autonomic outflow. This observation is interesting when considered with the finding that the right sympathetic nerves to the heart contain mainly chronotropic fibres while those on the left contain mainly inotropic fibres (Randall & Rohse, 1956; Linden & Norman, 1969). The data in the present study suggest that, assuming a symmetrical distribution of sympathetic fibres to other structures, the number of chronotropic neurons is approximately equal to the number of inotropic neurons.

An unexpected finding in this study was that the number of ILN neurons was found to be significantly greater in the males than in the females. This difference cannot be ascribed to a difference in body weight or in length of the ILN as no difference was apparent between the mean weights of animals or the mean lengths of the ILN in the two sexes (cf. Table 5). As no information was available on the ages of these animals, it is possible that a difference in the body weights of cats of the two sexes was not demonstrated because some of the animals in the sample were not fully developed. This possibility is given substance by the demonstrated sex difference in weights of adult cats (Latimer, 1936). The interpretation of these results would then be that the greater number of ILN neurons in the male is related to the statistical expectation of a greater body mass in the fully developed animal. The finding of a sex difference in the number of ILN neurons

suggests that the total number of neurons in the central nervous system is greater in the male. However, it is possible that this difference is limited to the sympathetic nervous system. This possibility is given substance by the demonstration that the number and size of neurons in sympathetic ganglia may be increased by the administration to newborn mice of a nerve growth factor isolated from the submaxillary gland of male mice and that the amount of nerve growth factor present in the gland is decreased by ablation of the testes and increased by administration of testosterone to female rats (Levi-Montalcini & Angeletti, 1964).

The data on neuron sizes are the first quantitative estimates of cell sizes of spinal autonomic neurons. These data are useful for comparison with data on surface areas of other spinal neurons. The areas of cat motoneurons in the anterior horn of L₅, S₁ and S₂ segments measured by Barr & Hamilton (1948) varied between 1650 and 2160 μm^2 , several times larger than the areas between 100 and 500 μm^2 found in ILN neurons. This clearly indicates that spinal sympathetic neurons present special difficulties for intracellular recording because of their relatively small size.

B. Localization of Cardioacceleratory Neurons in the ILN

Selective electrical stimulation of histologically verified sites on the right side of spinal segments T₁ to mid-T₈ elicited cardioacceleration and arterial hypertension in cats in which the adrenal

glands were removed, the cervical vagi were cut and the spinal cord was transected at C₇. The responses began with mean latencies of less than 3 sec and reached a maximum value at about 20 sec. These temporal characteristics are similar to those observed during electrical stimulation of cardioacceleratory nerves in the dog (Warner & Cox, 1962). On termination of the stimulus, heart rate and arterial pressure returned slowly to pre-stimulus levels. It is suggested that the responses persisted because in these animals activation of cardiodepressor mechanisms by baroreceptor stimulation could not be effective because the vagi had been cut bilaterally and the spinal cord had been transected at C₇, above the level of the sympathetic outflow (Gaskell, 1886).

The possibility was considered that the cardioacceleration observed during stimulation of the ILN was secondary to the pressor response. This possibility is unlikely in view of the finding that artificially induced arterial hypertension fails to elicit a change in heart rate in the isolated dog heart-lung preparation (Patterson, Piper & Starling, 1914). In addition, in two cats, administration of propranolol (2 mg/kg intravenously), a beta-adrenolytic agent known to eliminate the cardioacceleration elicited by electrical stimulation of the right stellate ganglion (Black, Duncan & Shanks, 1965), completely abolished the cardioacceleratory response to stimulation of the ILN without abolishing the pressor response.

As it has been demonstrated that changes in heart rate and arterial pressure occur during muscle contraction (Essex, Herrick, Baldes & Mann, 1939; Freyschuss, 1970 a, b; Adams, Baccelli, Mancina

& Zanchetti, 1971) the possibility that the cardiovascular responses observed were secondary to changes in motor activity was considered, but was excluded because the administration of gallamine triethiodide did not significantly alter the magnitude of the cardioacceleratory responses and because overt movements were not observed during stimulation.

The possibility that some of the responses obtained during stimulation of the spinal cord were due to activation of ascending or descending cardioacceleratory pathways must be entertained. This possibility, however, is very unlikely because the responses could not be elicited during stimulation of segment C₈ and cardioacceleration could not be elicited caudal to the middle part of segment T₈ despite the persistence of the arterial hypertensive response. In addition, the sites stimulated were identified as being distinctly separated from known cardiovascular pathways in the spinal cord (Illert & Seller, 1969). Furthermore, it was shown that stimulus spread did not contribute to the change observed because a displacement in the position of the tip of the electrode 0.5 mm dorsoventrally or mediolaterally resulted in disappearance of the physiological responses. Finally, it has been demonstrated that in the region of a unipolar stimulating electrode the power falls off exponentially with distance from the tip (Stark, Fazio & Boyd, 1962), and that 90% of the power is dissipated within 100 μ m of the tip (Krnjević, Randić & Straughan, 1966). It appears, then, that the observed effects were due to activation of

neurons in the ILN only.

The relation between stimulus frequency and magnitude of the cardiovascular responses demonstrates that for a given change in frequency the magnitudes of the heart rate and arterial pressure responses are greatest over the range of 0-5 and 0-20 Hz, respectively. This suggests that there probably is a physiologically optimal range of discharge frequencies of cardioacceleratory and vasopressor fibres where changes in heart rate and arterial pressure may be brought about by minimal changes in frequency. As it has been demonstrated (Folkow, 1952) that the normal discharge rate of vasopressor fibres is 1-2 per sec, which is at the lower limit of this optimal range, an increase, of the discharge rate of 10 impulses/sec from this normal rate can produce a nearly maximal response. The frequencies required in these experiments to produce maximal responses were greater than the 10-15 Hz found by Lindgren & Manning (1965) during electrical stimulation of the right stellate ganglion in the cat. This difference may be accounted for by two factors. One is that there may be a difference in the relation between the responses and stimulus frequency during activation of pre- and postganglionic neurons. The other is that the intravenous infusion of noradrenaline in our experiments may have altered the relation between the response and the stimulus frequency.

An interesting observation in some of the experiments was that much greater infusion rates of noradrenaline (up to 700 $\mu\text{g}/\text{min}$) were required to maintain the mean arterial pressure above 100 mm Hg than

were required to maintain the mean pressure at 90 mm Hg, and that at the higher infusion rates the magnitudes of the responses were greatly reduced and sometimes eliminated. In addition, it was observed that at the higher infusion rates the body temperature of the animals could be maintained above 37 °C throughout the experiment without the use of the external heating apparatus, suggesting that the production of heat was increased. As it has been demonstrated that noradrenaline has a calorogenic action (Spoelstra, 1963) the most likely explanation for the decreased magnitude of the cardioacceleratory and arterial pressor responses is that the metabolic rate was elevated to such an extent that the tissue oxygen requirements exceeded the local oxygen supply (Waters & deSuto-Nagy, 1950) and this local hypoxia led to decreased effectiveness of the noradrenaline released by activation of sympathetic fibres (Grandpierre, Frank, Arnould & Bouverot, 1954). This local hypoxia would also account for the much greater infusion rates of noradrenaline required to maintain the mean arterial pressure above 100 mm Hg as the effectiveness of circulating noradrenaline would also likely be reduced.

C. Descending Connections between the Medulla and Spinal Cardioacceleratory Neurons

Evoked responses were recorded from histologically identified sites within specific medullary structures during selective electrical stimulation of cardioacceleratory sites in the intermediolateral

nucleus (ILN) of the cat. The few responses observed at sites in non-nuclear regions might have been recorded from neurons located beyond the normal limits of their respective nuclei or been due to small variations in the positions of the nuclei in the medulla.

To determine whether the results may be used to identify the sites of origin of descending fibres projecting to cardioacceleratory neurons in the ILN it is first necessary to establish whether the responses recorded from the medulla represented synaptically or non-synaptically elicited activity and whether they were evoked orthodromically or antidromically.

Four criteria are commonly applied to distinguish synaptic from non-synaptic activity in experiments involving recording electrodes of the size used in the present series. One is based on the number of spikes observed for each stimulus pulse. A nerve fibre usually responds only once to a single stimulus of duration less than 0.5 msec whereas a single synchronous volley of impulses delivered over a presynaptic pathway often evokes a burst or train of spikes in the postsynaptic neuron (Bianchi, 1971). Repetitive responses to a single stimulus pulse to the spinal cord were not observed in the present series of experiments, suggesting that the evoked responses were not synaptically elicited.

A second criterion relates to the effects of the intensity of the stimulus on the latency of the response. When transmission involves a synapse the latency of the response varies with the intensity of the stimulus pulse, but axonal conduction is characterized by a response with a latency which is independent of the strength of the stimulus

(Bianchi, 1966). In the present study the latencies of the responses were not affected by changes in stimulus intensity, adding further support to the suggestion that the responses were not synaptically elicited.

The difference in the refractoriness of the synapse and of the axon provides a third means of testing whether a synapse is involved in the transmission of the evoked activity. Synaptically evoked activity fails to respond to every stimulus pulse at a frequency of less than 200 Hz (Miura & Reis, 1969) whereas antidromically evoked activity has an f_{50} of around 800 Hz (Crill & Reis, 1968); persistence of a response with each stimulus pulse at a frequency of 200 Hz is usually accepted as an indication that the response is not synaptically evoked (Porter, 1963; Wolstencroft, 1964; Car & Jean, 1971). All responses in the present study followed frequencies greater than 200 Hz. Therefore, it is very unlikely that the activity resulting from the stimulus to the spinal cord was transmitted across a synapse.

Finally, the fourth criterion for distinguishing between synaptic and axonal conduction is based on the fact that synaptic transmission is more susceptible to hypoxia and barbiturate anaesthesia than is axonal transmission (Miura & Reis, 1969). Synaptically elicited responses from the medulla have been observed to be abolished within 45 sec of the onset of hypoxia (Miura & Reis, 1969) while the antidromic fibre response to electrical stimulation of the medulla persisted for at least 90 sec (Crill & Reis, 1968). In addition, monosynaptic transmission in the medulla has been demonstrated to be eliminated following the intravenous administration of 5 mg/kg of sodium pentobarbital to a cat anaesthetized with chloralose (Miura & Reis, 1969) although the

antidromic fibre response remained unaffected (Crill & Reis, 1968). As the evoked responses in the present study remained unchanged after more than 90 sec of hypoxia and as they persisted following the administration of amounts of barbiturate anaesthesia four times as great as that used to eliminate monosynaptic transmission in the medulla it can reliably be concluded that in the present study transmission of the evoked responses between stimulating and recording electrodes was along axons only and that no synapses were involved.

Support for the possibility that the evoked responses were antidromic would be provided if it could be demonstrated that the responses in the present study were recorded from cell bodies rather than from fibre tracts. The fact that the responses were recorded almost exclusively from regions in the medulla corresponding to specific nuclei (cfr. Figure 23 and Table 9) suggests that they were recorded from groups of cell bodies in the medulla. In addition, the shift of the peak latency as a function of depth of the recording electrode, a characteristic of the responses observed in the present study, is not a property of the fields recorded perpendicularly to nerve tracts in the central nervous system, such as the pyramidal tract (Patton & Amassian, 1960). It is, however, compatible with the response recorded from groups of neurons, as in the hypoglossal N. (Lorente de Nó, 1947), the hippocampus (Cragg & Hamlyn, 1955) and the cerebral cortex (Chang, 1951), or from single spinal motoneurons (Nelson & Frank, 1964).

The results demonstrate, therefore, that the responses recorded from the medulla represent antidromic invasion of cell bodies elicited by activation in the spinal cord of the axons of these medullary neurons. The possibility remains, however, that the fibres activated in the spinal cord travelled close to the ILN but terminated on non-autonomic neurons in another part of the cord. The likelihood of the stimulus to the spinal cord spreading to adjacent fibres because of the use of unipolar electrodes is a particularly important factor to consider because of the extremely small cross-sectional area of the ILN and because of the close proximity of the ILN to fibres running in the white matter adjacent to the lateral horn. Therefore, to determine whether the responses recorded from the medulla were due to the stimulus spreading to non-autonomic fibres near the ILN, the effects on the responses of slight movement of the stimulating electrode were determined. The fact that the responses disappeared upon movement of the spinal cord electrode 0.5 mm strongly suggests that the stimulus was applied selectively to the ILN. In addition, support for the existence of a small stimulus field is that in the region of a unipolar stimulating electrode the power falls off exponentially with distance from the tip (Stark, Fazio & Boyd, 1962) and 90% of the power is dissipated within 100 μ m of the tip (Krnjević, Randić & Straughan, 1966). It is likely, therefore that the stimulus was applied to a very small region in the ILN. Finally, the fibres passing close to the lateral horn which might be activated by stimulus spread are propriospinal and consequently are

not connected directly with supraspinal structures (Crosby, et al., 1962; Riley, 1960). To conclude, the possibility can be rejected that the evoked responses recorded from the medulla were due to activation of non-autonomic fibres passing close to the ILN.

Therefore, as the evoked responses in the present study were elicited non-synaptically, as the properties of the responses were characteristic of those recorded from groups of cell bodies and as the stimulus was applied selectively to the ILN, it is concluded that the medullary evoked responses were recorded from the cell bodies of terminals activated in the ILN and that the structures in the medulla from which the responses were recorded project monosynaptically to neurons in the ILN.

As the techniques employed thus provide a reliable method for identifying the sites of origin of descending autonomic fibres which project directly to sympathetic preganglionic neurons the results of this part of the investigation will now be discussed. Sites in the medulla from which evoked responses were recorded correspond anatomically to several discrete structures. These include the raphe NN., the N. medullae oblongatae centralis subnuclei dorsalis and ventralis, the N. lateralis reticularis, the N. paramedium reticularis, the N. gigantocellularis, the inferior olivary N. and the N. parvocellularis (cfr. Table 9). These findings are the first electrophysiological demonstration of a monosynaptic connection between specific brain stem structures and sympathetic preganglionic neurons.

These results are also consistent with the literature surveyed in the historical review because all the structures implicated in this study have been previously shown to be involved with regulation of autonomic activity: the raphé NN. and N. lateralis reticularis have been implicated by Dahlström & Fuxe (1965) who demonstrated that the tryptaminergic and noradrenergic neurons in these nuclei project to neurons in the spinal cord including those in the lateral horn; the N. paramedium reticularis, the N. medullae oblongatae centralis, the N. gigantocellularis, the N. lateralis reticularis and the N. parvocellularis are sites of termination of primary or secondary cardiovascular afferent fibres (Crill & Reis, 1968; Miura & Reis, 1969; Biscoe & Sampson, 1970 a, b); the inferior olivary N. is in a descending cardiovascular pathway from the posterior hypothalamus to the ILN (Smith, 1965). The few evoked responses recorded from the N. pontis centralis caudalis are consistent with other information in the literature: this structure has been implicated in a descending vagal pathway to the heart (Achari, Downman & Weber, 1968) but the work was reported only in abstract form and evidence of accurate localization of electrode tips was not presented. An alternative explanation which might account for the responses from this nucleus is that as it is the rostral continuation of the N. gigantocellularis the responses observed may have been recorded from neurons in the rostral N. gigantocellularis. A similar explanation might account for the responses recorded from the NN. interfascicularis hypoglossi and retroambiguus as these

structures are located in close anatomical association with the N. paramedium reticularis and the N. medullae oblongatae centralis, respectively, structures from which many responses were recorded.

Therefore, it has been demonstrated that the medullary input to sympathetic preganglionic neurons in the spinal cord originates from discrete nuclear structures. Although each structure identified in the present study has been previously implicated in central mechanisms regulating autonomic activity, only this study provides information on the specific nuclei which project monosynaptically to sympathetic preganglionic neurons. The structures which receive primary or secondary afferent fibres and which project directly to ILN neurons are the N. paramedium reticularis, the N. gigantocellularis, the N. medullae oblongatae centralis and the N. parvocellularis. Because each of these structures receives first or second order fibres from the periphery and because each projects monosynaptically to ILN neurons, it is concluded that reflex pathways involving these structures can now be traced from the peripheral receptors, through the central nervous system, to the final neurons in the reflex arcs.

Evoked responses were noticeably absent from other medullary structures previously implicated in central control of the cardiovascular system. These include the N. of the tractus solitarius, implicated as a primary cell station of afferent cardiovascular fibres, the N. intercalatus, implicated in a descending cardiovascular pathway from the hypothalamus, and the dorsal N. of the vagus and the N. ambiguus, implicated as sites of origin of efferent vagal fibres. The most likely

explanations for the failure to observe responses in these nuclei are that either these structures are located on the afferent side of the reflex arc and therefore project to other structures in the brain stem, as is likely the case with the N. of the tractus solitarius and the N. intercalatus, or that their effects are mediated exclusively via the vagi, as is likely the case with the dorsal N. of the vagus and the N. ambiguus.

The latencies of the evoked responses varied from structure to structure, the mean latencies for each structure ranging from 1.03 to 1.97 msec. With a mean conduction distance of 95 mm, measured in three cats as the distance between the obex and the mid-T₂ level, the conduction velocities were calculated from the mean latency for each structure. These values ranged from 48.2 to 92.2 m/sec. These are the first calculations of conduction velocities of central autonomic fibres based on recordings within the central nervous system. Coote & Downman (1966) have reported that electrical stimulation of spinal afferent nerves produces short latency responses of 10 - 14 msec in the inferior cardiac nerve in the cat. However, as neither conduction distances nor the number of synapses likely to be involved were described by Coote & Downman, one cannot judge how their results compare with those obtained in the present series of experiments. More recently, Gootman & Cohen (1971) calculated the conduction velocity in the spinal cord to be 5 m/sec for the evoked response recorded in the splanchnic nerve during electrical stimulation of the descending autonomic tracts

in the ventrolateral white matter of the spinal cord. The difference between these results and those of the present study might be accounted for on the basis of a difference in the populations of neurons involved because it is possible that the supraspinal structures projecting to sympathetic preganglionic neurons in the splanchnic nerves (spinal levels T₅ to T₉; Reighard & Jennings, 1935) are different from those projecting to the most rostral part of the ILN in T₂ and that the fibres mediating different autonomic responses conduct at different velocities.

The bilateral representation of autonomic pathways from the medulla was also investigated in the present study. It has been suggested that the cell assemblies in the medulla regulating heart rate are more abundant on the right side than on the left and that most descending fibres from these structures are ipsilateral. Chai & Wang (1962) observed that stimulation on the right side of the medulla produced primarily an increase in heart rate while stimulation on the left produced mainly an increase in contractile force. The demonstration that stimulation of sympathetic nerves to the heart on the right side (Randall & Rohse, 1956; Mizeres, 1958) and of the ILN on the right side (see RESULTS, part B) elicits a greater change in heart rate than stimulation on the left suggests that the pathways regulating heart rate are found primarily on the right side and that little crossing-over occurs. To determine whether cardioacceleratory neurons in the ILN receive input from the contralateral medulla recordings were made from the left side of the medulla during electrical stimulation of cardioacceleratory sites in the ILN on the right side. Twenty-seven

penetrations yielded four sites from which evoked responses were observed. These results demonstrate that crossing-over of descending autonomic fibres occurs between the medulla and ILN neurons. However, the paucity of sites in the contralateral medulla supports the suggestion that the number of fibres which decussate is small and that mechanisms regulating heart rate are represented primarily on the right side.

As the principal objective of this investigation was to identify the sites of origin in the medulla of descending fibres which terminate specifically on cardioacceleratory neurons in the ILN attempts were made to determine the effects on heart rate and arterial pressure of electrical stimulation of 81 of the medullary sites from which evoked responses were recorded. Cardioacceleratory responses were localized mainly to sites in the N. lateralis reticularis and the N. parvocellularis. Sites from which cardiac slowing was elicited were localized mainly to the raphe NN., the N. paramedium reticularis and the N. medullae oblongatae centralis subnucleus ventralis. On the basis of the specificity of the cardioacceleratory response to stimulation of the ILN, the antidromic nature of the responses recorded from the medulla, the distribution of sites of recording within specific medullary nuclei and the selectivity of cardioacceleratory and cardioinhibitory responses almost exclusively to a few structures, it is concluded that the N. lateralis reticularis and the N. parvocellularis give rise to descending fibres which are excitatory to cardioacceleratory neurons in the ILN and that the raphe NN., the N. paramedium reticularis and the N. medullae oblongatae centralis

subnucleus ventralis give rise to descending fibres which are inhibitory to cardioacceleratory neurons in the ILN. It is possible that although the terminals of the descending inhibitory fibres were activated in the ILN their inhibitory effect on sympathetic neurons was mediated by interneurons. If this were so one would expect to observe cardiac slowing during electrical stimulation of the ILN at low voltages because neuron terminals have a lower threshold to stimulation than do cell bodies (Renshaw, 1940). As cardiac slowing was not observed during electrical stimulation of the ILN at low voltages it is suggested that interneurons were not involved in mediating the inhibitory response.

Arterial hypotension was elicited by stimulation of 39 sites in the medulla. The structures from which a decrease in pressure was most readily elicited were the raphe NN., the N. paramedium reticularis and the N. medullae oblongatae centralis subnucleus ventralis. As these were also the structures from which cardiac slowing was most readily elicited it is suggested that the two responses are mediated by similar mechanisms. Increases in arterial pressure were less frequently observed. Stimulation of the structures from which cardioacceleration could be reliably elicited led to inconsistent changes in arterial pressure. Three possible explanations might account for these observations. The first is that the parameters of stimulation in these experiments were such that they could not excite units mediating increases in arterial pressure. This possibility is very unlikely because using the same parameters of stimulation other cardiovascular responses were readily elicited. A second possibility is that in response to electrical stimulation of some of the sites in the medulla vasodilation and vaso-

constriction were occurring simultaneously and therefore the observable effect on arterial pressure was that no change occurred. As it has been demonstrated that electrical stimulation of specific structures in the central nervous system elicits changes in blood flow leading to pooling of blood in skeletal muscles at the expense of blood flow in other vascular beds (Rushmer, Smith & Franklin, 1959; Smith, Rushmer & Lasher, 1960) it is possible that a similar phenomenon occurred in the present study. A third possibility which might account for the observation that arterial hypertension was not reliably elicited from the structures from which cardioacceleration could be produced is that the mechanisms eliciting the two responses may be mediated by distinctly separate mechanisms.

With regard to the structures in the medulla from which no cardiovascular responses were elicited by electrical stimulation, it is likely that the terminals of their neurons projected onto cells mediating an autonomic function other than cardioacceleration or arterial hypertension. This is a likely possibility because it has been demonstrated that there is an overlapping of regions in the spinal cord mediating autonomic functions (Gellhorn et al., 1946) and therefore it is quite likely that neurons of a non-cardioregulatory function were among those within the field of stimulation.

In addition to identifying the structures in the medulla which project directly to ILN neurons and which therefore play a rôle in control of sympathetic activity, it is interesting to speculate on the conditions under which each structure is called into play. It is possible that the central mechanisms regulating the cardiovascular system are organized in

such a way that the reflex arcs involved in vegetative responses, generally localized to the medulla, and those involved in cardiovascular adjustments as part of more complex patterns of adaptation, such as occur during exercise, fear and sleep, each project to final common structures in the medulla mediating the appropriate responses. For example, all reflex arcs mediating cardioacceleration might project to one structure in the medulla which gives rise to the final common excitatory pathway to cardioacceleratory neurons in the spinal cord. This possibility is consistent with the currently accepted concept of the organization of central mechanisms regulating the cardiovascular system in which all afferent and supramedullary activity is integrated in the medulla and then transmitted to the spinal cord via the final common excitatory or inhibitory pathway (cfr. Figure 2). However, as it has been demonstrated in the present study that cardioacceleratory neurons in the ILN receive an excitatory input from two medullary structures and an inhibitory input from three medullary structures it is concluded that the functional organization of reflex arcs in the medulla does not involve a convergence onto one final common pathway from the medulla to the spinal cord.

The corollary to this conclusion should also be considered: that sympathetic preganglionic neurons receive inputs from more than one excitatory and one inhibitory structure. This organization is similar to the multiplicity of inputs to alpha motoneurons in the spinal cord (Mendell & Henneman, 1971). The suggestion of a convergence of spinal and supraspinal inputs onto ILN neurons has been suggested previously by Mannard & Polosa (1971). However, as they were recording single

unit activity of ILN neurons they were unable to eliminate the possibility that the convergence was onto a final pathway which then projected to ILN neurons, the present study is the first demonstration that convergence occurs at sympathetic preganglionic neurons and therefore that at least some integration of cardiovascular reflexes occurs at the spinal level.

Since convergence of autonomic pathways occurs at the level of the preganglionic neurons in the spinal cord it is likely that each structure projecting to these neurons is activated during conditions which are unique to the reflex arc of which it is a part. For example, investigations of the N. paramedial reticularis using lesion methods or electrical stimulation have demonstrated its rôle in the vasomotor components of the carotid sinus reflex (Share, 1965; Wang & Chai, 1967), in the depressor responses elicited by electrical stimulation of the forebrain (Löfving, 1961) and in pathways from the cerebellum involved in orthostatic reflexes (Reis & Doba, 1972). In addition, the raphe NN. have been associated with maintaining sleep (Jouvet, 1967) and it has been suggested that a polysynaptic projection from the carotid sinus nerve to these nuclei (Miura & Reis, 1969) mediates the effects of the carotid sinus and carotid body on sleep and wakefulness (Bartorelli, Bizzi, Libretti & Zanchetti, 1960; Baust & Heinemann, 1967). Finally, as the N. medullae oblongatae centralis receives input from the nucleus of the tractus solitarius (Morest, 1967), and the carotid sinus nerve (Miura & Reis, 1969) this structure may mediate the effects of carotid sinus nerve activity on antidiuretic hormone release (Share, 1965;

Rothballer, 1963) and on EEG synchronization (Bonvallet, Dell & Hiebel, 1954; Magnes, Moruzzi & Pompeiano, 1961).

However, it is not yet possible to identify the specific function of each medullary structure in the integration of cardiovascular reflexes. A knowledge of the types of activity integrated at each cell station requires information on the anatomical arrangement of the structures involved as well as the exact homeostatic rôle of the individual reflex arcs. The present investigation has contributed to the understanding of cardiovascular reflexes by identifying specific medullary structures which project monosynaptically to cardioacceleratory neurons in the ILN and by establishing a functional significance with regard to cardiovascular regulation to each of these structures.

D. CONCLUSIONS

The thoraco-lumbar intermediolateral nucleus (ILN) was observed in the cat between spinal segments C₈ - T₁ and L₄, and was in the lateral horn in all segments except T₁ - T₂ and L₁ - L₂ where neurons were scattered throughout finger-like projections of the grey matter. Total neuron counts for individual cats ranged from 32,790 to 53,340. The mean counts on the left and right sides were not statistically different. It was demonstrated, however, that the mean counts in the males were significantly greater than those in the females. The number of ILN neurons varied from segment to segment with the highest counts occurring in segments T₁ - T₂ and L₃ - L₄. Quantitative estimates of cell sizes were also obtained from one male cat; the modal cross-sectional area was 290 μm^2 .

In addition, it was demonstrated that in adrenalectomized cats with bilateral vagotomy and spinal transection at the C₇ level selective electrical stimulation of histologically verified sites within the ILN on the right side of the spinal cord in segments T₁ to mid-T₈ elicited cardioacceleration and arterial hypertension. The responses were not affected by the administration of a paralyzing agent. Administration of a beta-adrenolytic agent abolished the cardioacceleratory but not the arterial hypertensive response.

As supraspinal reflex input to the heart was eliminated, the animals were adrenalectomized and the cardioacceleratory response was not secondary to motor activity or to the arterial pressor response it was concluded that the cardioacceleration was due to direct activation of sympathetic preganglionic neurons and that cardioacceleratory neurons in the ILN are distributed between segments T₁ and mid-T₈. Furthermore, it was demonstrated that cardioacceleratory responses to stimulation of the ILN on the right side were significantly greater than those on the left, suggesting that cardioacceleratory neurons are more abundant on the right side. Data were also obtained on the relation between the stimulus frequency and the magnitude of the response, demonstrating that there is a physiologically optimum range of discharge frequencies where changes in heart rate and arterial pressure may be brought about by minimal changes in frequency. These data also provide information on the probable firing frequency of sympathetic preganglionic neurons at various levels of heart rate and arterial pressure.

During electrical stimulation of cardioacceleratory sites in the ILN on the right side of segment T₂ evoked responses were recorded from histologically verified sites in the medulla. The responses were elicited non-synaptically because each consisted of a single deflection with a latency independent of the stimulus intensity, each followed frequencies of greater than 200 Hz and each was unaffected by hypoxia and by an overdose of a barbiturate anaesthetic. The responses were recorded from groups of cell bodies because they showed the characteristic

shift of peak latency with distance from an active region typical of recordings from cell groupings and because the sites of recording were histologically identified within specific medullary nuclei. The stimulus likely activated descending autonomic fibres selectively because the sites of stimulation were histologically verified to be within the ILN, because displacement of the stimulating electrode of 0.5 mm dorsoventrally or mediolaterally eliminated the response from the medulla and because fibres adjacent to the ILN are propriospinal. On the basis of this information it was concluded that the evoked responses represented antidromic invasion of the cell bodies of terminals activated in the ILN and therefore that the medullary structures from which evoked responses were recorded project monosynaptically to sympathetic preganglionic neurons in the ILN. Electrical stimulation of sites from which evoked responses were recorded elicited cardioacceleration from the N. lateralis reticularis and the N. parvocellularis and cardiac slowing from the raphé NN., the N. paramedium reticularis and the N. medullae oblongatae centralis subnucleus ventralis. Stimulation of other medullary sites produced no changes in heart rate. Based on the specificity of the cardioacceleratory response to stimulation of the ILN, the antidromic nature of the evoked responses recorded from the medulla, the distribution of sites of recording within specific medullary nuclei and the localization of cardioacceleratory and cardioinhibitory responses to

specific structures it is concluded that the N. lateralis reticularis and the N. parvocellularis give rise to descending fibres which are excitatory to cardioacceleratory neurons in the ILN and that the raphe NN., the N. paramedium reticularis and the N. medullae oblongatae centralis subnucleus ventralis give rise to descending fibres which are inhibitory to cardioacceleratory neurons in the ILN. Arterial hypotension was elicited by stimulation of the same structures as cardioinhibition, suggesting that the two responses are mediated by similar mechanisms. Stimulation of the structures from which cardioacceleration was elicited led to inconsistent changes in arterial pressure, suggesting that these responses are mediated by different mechanisms. Structures from which no changes in heart rate or arterial pressure were elicited are likely involved in reflex arcs regulating non-cardiovascular activity.

It was also demonstrated that the functional organization of medullary mechanisms regulating the cardiovascular system does not involve convergence of integrated activity to a final common pathway to the spinal cord because the results identified several cardioregulatory structures in the medulla which project directly to cardioacceleratory neurons in the ILN.

Finally, this is the first demonstration of a convergence of inputs directly onto sympathetic preganglionic neurons and therefore that at least some integration of cardiovascular reflexes occurs at the spinal level.

SUMMARY

1. Serial transverse sections of the spinal cord of eight adult cats were stained with thionin and examined to determine the topography of the thoraco-lumbar intermediolateral nucleus (ILN) and the numerical segmental distribution of its neurons. The ILN was found to extend from the junction of segments C₈ and T₁ to segment L₄, and was observed in the lateral horn of the grey matter except in segments C₈ - T₁ and L₁ - L₂. In these segments the neurons were observed scattered throughout finger-like projections of the grey matter. The number of ILN neurons varied from section to section, a characteristic distribution of ILN neurons referred to as "beading". The segmental distribution of ILN neurons was also variable, with the greatest number of neurons at the levels of the rostral thoracic (T₁ - T₂) and of the middle lumbar (L₃ - L₄) segments. The total number of neurons in each of the cats studied ranged from 32,790 to 53,340. The mean counts on the left and right sides were not significantly different. However, the mean counts in the males were significantly greater than those in the females. The modal cross-sectional area of ILN neurons was estimated to be approximately 290 μm^2 .

2. Selective electrical stimulation of the ILN, with histological localization of the sites of stimulation, was attempted in cats anaesthetized with alpha chloralose (80 mg/kg I.V.). Stimulation on

the right side of segments T₁ to mid-T₈ elicited short latency cardioacceleration and arterial hypertension in 20 cats in which the adrenal glands were removed, the cervical vagi were cut and the spinal cord was transected at C₇. The administration of a beta-adrenolytic agent (propranolol, 2 mg/kg I.V.) abolished the cardioacceleratory response but left the pressor response intact. In addition, the administration of a paralyzing agent (gallamine triethiodide, 5 mg/kg I.V.) did not alter the magnitudes of the cardiovascular responses.

3. The magnitudes of the cardioacceleratory responses elicited by stimulation of the ILN on the right side were significantly greater than those elicited by stimulation on the left.

4. Data were obtained on the relation between the stimulus frequency and the magnitude of the response, providing information on the probable firing frequency of sympathetic preganglionic neurons at various levels of heart rate and arterial pressure. For a given change in stimulus frequency the magnitude of the cardioacceleratory response were greatest over the range of 0-5 Hz; the change in magnitude of the pressor response was greatest over the range 0-20 Hz.

5. During electrical stimulation of histologically verified cardioacceleratory sites in the ILN on the right side of the spinal cord in 22 cats, evoked responses were recorded from 266 sites within specific medullary structures. The responses were likely transmitted non-

synaptically because they consisted of a single deflection with a latency which was independent of the intensity of the stimulus to the spinal cord, they followed stimulus frequencies of greater than 200 Hz and they were not affected by hypoxia or by an overdose of a barbiturate anaesthetic. The responses were recorded from groups of cell bodies because the sites from which they were recorded were localized almost exclusively to nuclear structures and because they showed the shift of peak latency when passing through an active area that is characteristic of groups of neurons but not of fibre tracts. The neurons from which responses were recorded in the medulla were those whose terminals projected onto ILN neurons because the sites of stimulation were histologically verified to be within this nucleus, displacement of the stimulating electrode 0.5 mm in any direction eliminated the response from the medulla, the size of the field of the stimulus around the tip of the electrode was small and because other fibres in the vicinity of ILN neurons are propriospinal and are therefore not associated directly with structures in the medulla. As the evoked responses were elicited non-synaptically, the responses were characteristic of those recorded from groups of cell bodies and the stimulus was localized to the ILN it is concluded that these responses represented antidromic invasion of the cell bodies of terminals activated in the ILN and therefore that the structures in the medulla from which responses were recorded project monosynaptically to sympathetic pre-ganglionic neurons in the ILN.

6. The structures in the medulla from which evoked activity was recorded include the raphé NN, the N. medullae oblongatae centralis subnuclei dorsalis and ventralis, N. lateralis reticularis, N. paramedium reticularis, N. gigantocellularis, N. parvocellularis and inferior olivary N. These findings are the first electrophysiological demonstration of a monosynaptic connection between specific brain stem structures and sympathetic preganglionic neurons.
7. As evoked responses were recorded from structures on both sides of the medulla during electrical stimulation of the ILN it was concluded that crossing-over of descending autonomic fibres occurs between the medulla and the ILN. However, the small number of sites in the contralateral medulla suggests that ipsilateral connections are more abundant.
8. Electrical stimulation of the medulla at sites from which evoked responses were recorded produced cardioacceleration at sites in the N. lateralis reticularis and the N. parvocellularis and cardiac slowing in the raphé NN., the N. paramedium reticularis and the N. medullae oblongatae centralis subnucleus ventralis. On the basis of the specificity of the cardioacceleratory response to stimulation of the ILN, the antidromic nature of the responses recorded from the medulla, the distribution of sites of recording within specific medullary nuclei and the localization of cardioacceleratory and cardioinhibitory responses almost exclusively to a few structures, it is concluded that the N.

lateralis reticularis and the N. parvocellularis give rise to descending fibres which are excitatory to cardioacceleratory neurons in the ILN and that the raphé NN., the N. paramedium reticularis and the N. medullae oblongatae centralis subnucleus ventralis give rise to descending fibres which are inhibitory to cardioacceleratory neurons in the ILN.

9. Arterial hypotensive responses were restricted almost exclusively to sites within the structures from which cardiac slowing was elicited suggesting that the two responses are mediated by similar mechanisms. Stimulation of the structures from which cardioacceleration was elicited, led to inconsistent changes in arterial pressure, suggesting that these responses are mediated by different mechanisms.

10. Structures from which evoked responses were recorded but from which no changes in heart rate or arterial pressure were produced by electrical stimulation are likely involved in reflex arcs regulating non-cardiovascular activity.

11. As it has been demonstrated that cardioacceleratory neurons in the ILN receive an excitatory input from two medullary structures and an inhibitory input from three medullary structures it is concluded that the functional organization of reflex arcs in the medulla does not involve a convergence of integrated activity onto one common final pathway from the medulla to the spinal cord.

12. This is also the first direct demonstration of a convergence of inputs onto sympathetic preganglionic neurons and therefore that at least some integration of cardiovascular reflexes occurs at the spinal level.

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